GROUP-LIVING AND VIBRATIONAL COMMUNICATION IN LARVAE OF THE AUSTRALIAN SAWFLY, *PERGA AFFINIS*

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Lynn Erin Fletcher

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GROUP-LIVING AND VIBRATIONAL COMMUNICATION IN LARVAE OF AN AUSTRALIAN SAWFLY, *PERGA AFFINIS*

Lynn Erin Fletcher, Ph. D.

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Group-living occurs throughout the Animal Kingdom. Its ubiquitous nature raises two fundamental questions: why do organisms do it, and how do they maintain their group structures? Answers to these questions shed light on the evolution of social living and from them, two principles emerge. The first principle relates to why organisms live with others. In order to evolve, grouping must provide a net fitness benefit to the individual members. In this way, group members attain a higher level of reproductive success than those living alone. The second principle relates to how organisms live in groups and here, communication is key. Groups are composed of separate entities and as such they require a mechanism to form and maintain a cohesive unit; communication provides such a mechanism. Additionally, communication provides a medium of exchange during competitive and cooperative interactions, both of which occur in group settings. It serves to minimize costs of association from competition while enhancing benefits that stem from cooperation.

For my dissertation, I investigated why and how larvae of the Australian sawfly, *Perga affinis*, live in groups. In light of the two principles, I examined both the benefits of group-living and the role of vibrational communication in their gregarious lifestyle. Chapter 4 contains experiments testing for benefits related to predation protection, thermoregulation, immune function, feeding efficiency, and pupation success. While grouping provided an overall survivorship advantage, the

main benefits stemmed from thermoregulation and feeding facilitation. Chapters 1-3 provide detailed studies of P. *affinis*' vibrational communication. In chapter 1, I characterized two signals, contractions and tapping, and tested whether the signals were competitive or cooperative in nature; both have cooperative functions. In Chapter 2, I examined tapping as a mechanism for cohesion between groups and separated larvae while also investigating differential levels of investment in the signal exchange. The exchange was analogous to the Raise-the-Stakes model of cooperation where groups gradually increased their investment according to the time spent signaling by the single larva. In chapter 3, I tested whether or not different tapping rates encoded for alternate signal meanings via a playback experiment.

BIOGRAPHICAL SKETCH

Lynn Erin Fletcher was born in Concord, MA with her twin sister, Karen. She spent the first 8 years of her life in MA where the woods around her home served as her favorite playground. Growing up in a family that loved the outdoors, she has many fond memories of swimming in lakes, biking in neighborhoods, sledding and skating on nearby hills and ponds, and picking apples in the local orchards. At the age of 8, her family moved from their 300-year old New England town to a new suburb in CA, celebrating its 5th anniversary. Before this move, Lynn had always loved animals and concerned herself with the affairs of nature through hours of play and observation. After the move, however, her interest in nature took on a sense of urgency. In CA she was surrounded by budding suburbs and dwindling hillsides, where despite the ever-growing human population, she experienced an acute sense of loss of community - the natural community.

While Lynn had always enjoyed school, she now immersed herself in her studies as she knew that a good education would enable her to be more effective at helping the natural world. Along with education, she engaged in activities such as founding an Earth Club, starting a school-wide recycling program, and volunteering with injured raptors at the Lindsay Museum. All of these activities provided a way of working towards her goal but she continually missed connecting with the goal's origin - being immersed in nature, like she had been as a child, through both observation and contemplation.

In the Fall of 1994, Lynn entered Mount Holyoke College as a Biology major. It is here where she met her first biology mentor, Dr. Susan Smith. Lynn took Susan's ornithology course that Spring and proceeded to take every course Dr. Smith offered, which included Animal Behavior. As animal behavior is all about observation and (scientific) contemplation, Lynn was thrilled to learn about this discipline! Dr. Smith

provided an important link as well since both her teaching and research were infused with a sense of respect and admiration for the organisms involved. Through Dr. Smith's example, Lynn saw a way of positively contributing to the natural world, realizing that both research and education could influence others in a beneficial way. As a senior, Lynn completed a thesis with Dr. Smith on a medium-intensity warning vocalization in black-capped chickadees and graduated Summa cum Laude in May of 1998.

After living in Washington DC for two years and working as a Science Assistant at the National Science Foundation, Lynn returned to her studies by starting a PhD program at Cornell University in the Neurobiology and Behavior Department. It is here that she met her second important mentor and advisor, Dr. Thomas Eisner. As one of the great naturalists of our time, Dr. Eisner has been, and continues to be, a great source of inspiration. Lynn will stay at Cornell University as a postdoctoral associate with Dr. Eisner for a year following her graduation.

To my Dad,

with great love and affection

ACKNOWLEDGMENTS

As with any major piece of work, many people have been involved, and collectively, they have made this dissertation possible. The first person is my advisor, Tom Eisner, whose inspiring work and love of the natural world brought me to Cornell to pursue my PhD. Tom has been an incredible mentor, offering nothing but positive encouragement since our first meeting. He and his wife, Maria, have also been extremely supportive of my Australian endeavors and I was even offered the use of Maria's steel-toed boots for my escapades into snake country! Jayne Yack played a key role in my hunt for a project; our work on cherry leaf-roller caterpillars hooked me onto larval communication and acoustics, at which point she encouraged investigation into the sawfly literature. Here, I read Peter Carnes' pioneering work on *Perga affinis*, which provided me with the necessary background material to begin my research. My committee members, Cole Gilbert, Kern Reeve, Ron Hoy, and Chris Clark, have been very helpful in discussing experimental plans as well as manuscripts that resulted from the studies. Rex Cocroft and Bob Grotsky were both instrumental in configuring the acoustic recording set-up and Françoise Vermeylen has been an incredible resource of statistical knowledge and advice during all of my data analysis. Two undergraduate students, Anita Tseng and Michael Robinson, provided invaluable assistance in collecting data from video recordings and experiments, saving me countless hours in front of a TV monitor! And the staff in NB&B, particularly Janis Strope and Terri Natoli, have been extremely helpful with all administrative matters. I would also like to thank my funding sources, NSF, Sigma Xi, and NB&B.

In Australia, I am grateful to John MacDonald and all the members of the Gurr lab at the University of Sydney at Orange, who hosted me for my first field season. Bill Foley, Ian Wallis, and Paul Cooper were instrumental in enabling me continue research at the Australian National University in Canberra. Martin Steinbauer and

Rex Southerland at CSIRO provided assistance in finding viable populations of larvae while also allowing me to collect individuals from their study site. The Department of Botany and Zoology generously provided me with desk-space, access to lab equipment, and use of the greenhouse facilities.

Friends and fellow graduate students have provided so much support as well as opportunities for fun during this whole process. Living at the Stewart Little Co-op was fantastic and provided an instant family of mice, including two dear friends, Carrie Brown and Debbie Gross. Jude Scarl, Laurieanne Dent, Kamal Shukla, Ann Marie McNamara, Shannon Olsson, Brian Johnson, Liz Tibbetts, Danielle Cholewiak, Katie Knight, Sheng-Feng Shen, Biz Turnell, Chris Wiley, Dan Fergus, and all the grad students in NBB have been wonderful friends as well as colleagues. In Australia, I had the honor of meeting and befriending many lovely people including Meredith Cosgrove, Mahmuda Begum, Jon Graftdyk, Kelli Gowland, and Andrea Leigh.

My family has been incredibly supportive and encouraging through out this entire endeavor. Both my Mom and sister visited me in Australia and my Dad, who couldn't travel at the time, always called regularly to hear about my life Down Under. And while in Ithaca (which, although in the US, is not that easy to reach!), Karen, Dad, and Steve have come to see me and I've had multiple visits from Mom and Rick as well as Uncle Jim and Lisa. They all seem excited about the sawflies too and although my research is not the easiest to explain to inquiring neighbors and friends, they gladly take up the task of promoting these larvae! While on medical leave, my entire family, including Steve who opened up his home in Carmel, were incredibly generous and provided so much love and support. I have so much gratitude for my family and their presence in my life.

Thanks to all of you who have been involved in this project and, of course, I offer a heartfelt thanks to the lovely sawflies who made everything possible!

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CHAPTER 1

VIBRATIONAL SIGNALS IN A GREGARIOUS SAWFLY LARVA (*PERGA AFFINIS*): GROUP COORDINATION OR COMPETITVE SIGNALING?*

Abstract

Group living confers both benefits and costs to the individuals involved. Benefits may include enhanced defense, thermoregulation, and increased foraging efficiency while costs often involve competition for resources such as food, shelter and mates. Communication provides a medium of exchange among individuals engaged in either cooperative or competitive interactions. The functional analysis of signals within groups therefore requires testing both cooperative and competitive functions, although the latter is infrequently done. In this paper, I study the use of two vibrational signals in a gregarious, processionary Australian sawfly larva, *Perga affinis*: tapping and contractions. Tapping involves striking the substrate with the sclerotized portion of the abdominal tail and a contraction is a fast, whole-body twitch, which is both tactile and vibrational in its transmission. For tapping, I first demonstrate that it is a form of communication, as tapping of one larva elicits tapping in another, and that it is transmitted through substrate vibrations. I then test whether the signal is mostly cooperative or competitive in nature by examining it in light of two hypotheses: 1) the Group Coordination hypothesis, stating that the signal functions to maintain group cohesiveness and 2) the Competitive Signaling hypothesis, stating that tapping serves as a competitive assessment signal between larvae while feeding. For contractions, I test only the group coordination hypothesis that they serve to coordinate and initiate group movement. Results support the group coordination hypothesis for each signal. While feeding, lone larvae (without potential competitors) were significantly more

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likely to tap than those in groups and this trend continued in non-feeding situations. Contractions regularly preceded periods of group movement during processions and were given with increasing frequency before departure from preforaging clusters. The vibrational signals in this processionary species likely function cooperatively to maintain group cohesiveness and coordinate movement.

Introduction

Group-living involves the act of balancing costs and benefits. Individuals may lessen costs by competing with other group members; alternatively, they may enhance the benefits of association through cooperation. Communication among group members serves as an important tool in either endeavor.

Communication for competitive purposes occurs in many group-living environments, such as those involving colonial insects (Reeve 1991; Tibbetts 2002), breeding congregations (Bourne 1992; Stein and Uy 2006) or foraging aggregations (Radford 2004b). Group-living animals also communicate for more cooperative purposes. Many organisms such as birds, dolphins, schools of fish or herds of elephants use signals and communication as a way to attract group members and to maintain group structure and cohesiveness while traveling (Moller 1976; Black 1988; Janik and Slater 1998; Langbauer 2000; Radford 2004a). Among insects, many species have gregarious larvae that engage in daily movement and they use a variety of communication modalities to maintain group structure such as pheromones, silken trails, and/or tactile cues (Fitzgerald 1995; Costa and Louque 2001; Ruf et al. 2001; Costa et al. 2004).

This paper investigates the signals used in communication by a gregarious, processionary Australian sawfly larva, *Perga affinis* (Pergidae: Hymenoptera). Like other processionary species, tactile cues appear to play an integral role in group

structure as larvae remain physically connected (and often overlapping with the tail of one around the head of another) at all times except when feeding; however, there is no evidence of pheromone trails and larvae do not produce silk. Instead of coupling tactile signals with these more commonly described modalities, behavioral observations suggest that *P. affinis* may use substrate vibrations (Evans 1934; Carne 1962). Vibrational communication is common among insects (Cocroft and Rodriguez 2005); however, the majority of work has focused on adults (i.e. duetting between the sexes and male-male competitive interactions) (Cokl and Doberlet 2003; Cocroft and Rodriguez 2005) or on communication between adults and their brood, as seen in wasps (Harding and Gamboa 1998; Savoyard et al. 1998; Cummings et al. 1999). A few studies have documented its presence and function among nymphs or larvae (Russ 1969; Hograefe 1984; Yack et al. 2001; Cocroft 2005; Fletcher et al. 2006). If backed by experimental evidence, larvae of *P. affinis* would provide an example of a processionary species using vibrational signals to maintain group cohesion and prevent fragmentation while processing.

Although it is easy to assume that any form of communication in a processionary species serves to promote group cohesion and integrity, it is important to consider alternative functions, especially given the context of group-living. As a gregarious species, *P. affinis* faces both the costs and benefits of group association and therefore one cannot rule out a potential competitive function of its signals. Many organisms that aggregate or form groups use signals to mediate competition for resources such as mates or food (e.g. Nelson and Fraser 1980; Guerra and Mason 2005). Among insects, acoustic signals are also involved in competitive situations. Several cases have documented larvae using acoustic signals to mediate competitive territorial disputes (Russ 1969; Yack et al. 2001; Fletcher et al. 2006) Alternatively, there are at least two cases involving larval and nymphal insects where acoustic

signaling serves a cooperative function by attracting siblings to a good food source (Hograefe 1984; Cocroft 2005). Therefore, I consider both competitive and cooperative hypotheses when investigating the function of a vibratory signal in *P. affinis*.

P. affinis has two acoustic signals: tapping and contractions. Tapping occurs when a larva strikes the substrate with the sclerotized portion of its abdominal tail and a contraction is a fast, whole-body twitch. In this paper I first describe and characterize the signals and also demonstrate that tapping is a form of communication transmitted through the substrate. I then examine their function in light of two hypotheses, a Group Coordination Hypothesis and a Competitive Signaling Hypothesis. For tapping, I test the coordination hypothesis that this signal promotes group cohesiveness by serving as a 'where are you?' or 'I am here' signal for larvae that become separated from the group. I also test the alternative hypothesis that tapping acts as a competitive signal between larvae for gaining access to a common food source (much like the territorial signal of *Drepana*, see Yack et al. 2001). This sets up 4 discriminatory predictions (see Table 1.1). Predictions 1 and 2 support the coordination hypothesis of a 'where are you?' or 'I am here' function since they predict lone individuals will tap more and have a lower latency to tap than those in groups. This is expected if tapping serves to promote finding and aggregating with other larvae. Prediction 3 is compatible with the competitive hypothesis by predicting that when feeding, a larva on a leaf is more likely to tap when others are present (and hence offer competition) than if it is alone. Prediction 4 is compatible with the coordination hypothesis because a predicted positional effect of tapping while in a group suggests a form of coordination or organization.

For contractions, since the signal is not given in a context that could be considered competitive (i.e. while accessing a shared resource), I only test the

Table 1.1 – Predictions of the alternative hypotheses for signal function

Predictions	Competitive Signaling Hypothesis	Group Coordination Hypothesis	Observed
Tapping			
P1: Larva taps more when alone than when in a group	-	+	+
P2: Lower latency to tap (i.e. taps sooner) when alone than in group	-	+	+
P3: When feeding, larvae on a leaf with at least one other individual are more likely to tap than a single larva on a leaf	+	-	-
P4: When in a group, there is a positional effect on the occurrence of tapping	-	+	+
Contractions			
P1: Larvae only contract when in a group (not alone)		+	+
P2: Contractions precede periods of group movement		+	+
P3: Positional effect in timing of or initiation of contraction		+	+

coordination hypothesis. Specifically, I examine whether or not contractions function to initiate and orchestrate group movement. Three predictions are tested: P1) larvae only contract when in a group and not when alone, P2) contractions precede periods of group movement and P3) there is a positional effect in the timing of or initiation of a contraction.

The above predictions for both signals are tested experimentally as well as through quantified observations. Results are discussed for each signal separately.

Methods

Natural History and Daily Patterns of *P. affinis* Larvae. Adult female sawflies lay clusters of 20-30 eggs in the leaves of several species of *Eucalyptus* in early April (Carne 1962). After about a month of incubation, larvae hatch together and emerge forming tight cycloalexic formations (a rosette pattern with heads pointed outward and the posterior abdomens pointed inward, see Jolivet et al. 1990) during the day and feeding on the leaf's perimeter at night. As the larvae grow, they maintain their nocturnal activity patterns but shift their daytime resting site to small twigs and eventually branches, where they form a cluster of overlapping bodies (no longer using cycloalexy). They remain gregarious for their entire larval stage (which lasts up to 6 months).

In their daily movement as nomadic foragers (Fitzgerald and Peterson 1988), the larvae move to different feeding and resting sites by processing as a group. The transition between the tight daytime clusters to the processions involves an intermediate stage that I have referred to as a preforaging cluster (a tight cluster 1-2 hours before the procession begins). It is during this stage that one begins to see an increase in activity and signaling behavior in the larvae.

Insects and Plants. Colonies of sawfly larvae *(Perga affinis*) were collected from Orange, NSW (2002) and the Australian Capital Territory (2003) and maintained on cuttings from their host plant. Colonies used for observations on preforaging and processionary behavior were kept indoors on a reverse light cycle (L:D 11:13 hours) at a constant temperature of 21°C. Larvae used for experiments on the tapping behavior were kept on branch cuttings in an outdoor shade house. All recordings and trials took place on host plant cuttings or branches, thus providing a natural substrate for observations.

Acoustic and Video Recordings. Preforaging and processionary recordings took place under dark conditions, with a red lamp and infrared light from the video camcorder to monitor behavior, while all tapping experiments were conducted during daylight hours. Vibrational signals and movements of the larvae were picked up by a phono cartridge placed on the substrate. The phono cartridge was attached to a Sound Devices MP-1 preamplifier that fed into a SONY Digital 8 DCR-TRV830 camcorder, thereby allowing simultaneous acoustic and visual information to be recorded. Separate samples of tapping and group constrictions were recorded with a BU Series Knowles accelerometer (model BU-1771) and an LEEM guitar transducer (connected to the preamplifier and the camcorder) for more precise analysis of signal spectral characteristics. Acoustic recordings used for characterization were made using Sound files of the tapping signal were imported as wave files to a PC and analyzed using Raven 1.2 software from the Cornell Laboratory of Ornithology.

Preforaging and Processionary Behavior. Ten colonies (ranging in size from 15-45 larvae) were monitored for the description and quantification of preforaging and processionary behavior.

Preforaging clusters are the same as tight daytime clusters but are defined in terms of their temporal proximity to the start of a procession (i.e. they are tight clusters

1-2 hours before larvae process to forage). To monitor them, I recorded the timing and relative group involvement (a few individuals, half the group, or the whole colony) of any signaling events prior to departure. This measured the frequency of signal use over time; it also enabled me to examine prediction 2 (P2) regarding the timing of contractions in relation to periods of group movement (see Table 1.1).

For the processionary behavior, I examined three procession events for each of six colonies. A procession event begins with waves of contractions that pass through the group followed by walking and ends when the group stops all movement. To test P3 for contractions and P4 for tapping (see Table 1.1), I also investigated the effect of larval position within the colony on the use of signaling during the procession event. For each colony I monitored three front, three middle, and three back-positioned larvae. A Mann-Whitney *U* test was used to compare the proportion of front versus back-positioned larvae per colony that tapped during procession events. A Wilcoxon-signed rank test examined the timing of contractions by comparing the proportion of times contractions moved from front to back individuals (null hypothesis, proportion = 0.5).

Function of Tapping. First, I tested to see if tapping serves as a form of communication and examined whether the signal is transmitted through substrate-borne or air-borne vibrations. Then, to examine the function of tapping, I tested a set of predictions based on two alternative hypotheses: the group coordination hypothesis (tapping aids in maintaining group cohesiveness by serving as a 'where are you?' or 'I am here' signal as well as coordinating movement when processing) and the competitive signaling hypothesis (tapping serves as a competitive signal between larvae gaining access to a common food source) (see Table 1.1). Tapping occurs in a variety of contexts (see Table 1.2), so I examined the function in three situations: single, lone larva versus a larva in a group (P1, P2), individual and grouped larvae

Table 1.2 Contexts of Signals

Context	Contractions	Tapping
Daytime tight clusters	Negligible	Negligible
Preforaging clusters	Yes	Yes
Processions	Yes	Yes
Foraging	No	Yes
Returning to colony	?	Yes

while foraging (P3) and larvae during processions (P4, see the Preforaging and Procession section of Methods). I tallied each prediction according to whether it supported (+) or did not support (-) a given hypothesis and then compared these scores with the observed outcome.

Tapping – A Form of Communication? If tapping serves as a signal in communication, then the tapping should elicit some type of behavioral response in the receiver (Bradbury and Vehrencamp 1998). To test this, I conducted a paired experiment with 20 experimental and control trials (both 20 minutes in duration). In the experimental set-up, two larvae were placed on opposite sides of a single eucalyptus branch, each equidistant (10.5 cm) from and facing the center cardboard divider (Figure 1.1). This set-up allowed larvae to 'hear' each other and receive vibrational cues through the substrate while preventing the transfer of any visual information. The typical propagation ranges for substrate vibrations are 30 cm to 2 m (Michelsen et al. 1982; Cokl and Doberlet 2003; Cocroft and Rodriguez 2005), so the larvae were well within receiving distance for this experiment.

The control trials had a similar set-up except the larvae sat on separate branches (placed on different tables 50cm apart from each other, see Figure 1.1). Larvae received neither visual information (due to cardboard blocks) nor vibrational

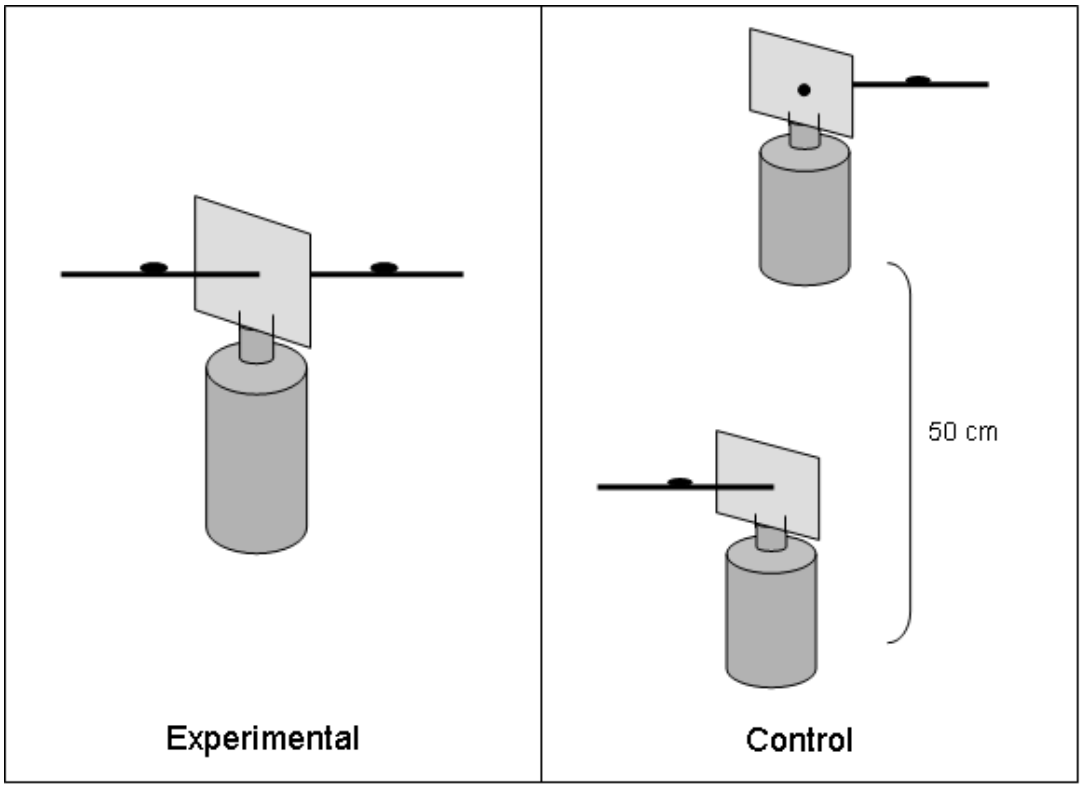


Figure 1.1 Experimental and control set-up for tapping experiment. For experimental trials, larvae sat on the same branch separated by a cardboard insert. For control trials, larvae sat on separate branches attached to different bottles that were a distance of 50 cm from each other. In both set-ups, the larvae were placed 10.5cm from the center divider.

cues through the substrate. The set-up, in addition to testing for communication, tested whether the signal was substrate-borne or air-borne. If tapping is perceived through substrate vibrations, the behavior of larvae between the control and experimental trials should differ. If the signal is air-borne, there should be little or no difference.

Data collected from the videotapes involved recording the start and end time (duration) of the following behaviours for each larva: tapping, walking, searching (lifting the head and 1st pair of thoracic legs off the substrate), and turning around. A Mathematica™ program developed by Dr. Kern Reeve (Cornell University) calculated the total time of tapping overlap (periods where one individual is tapping and the second larva joins in) for each trial. The program, using bootstrap analysis, calculated both the observed and expected overlap (if the overlap occurs randomly), which was

then used in a Paired *t* test. As a second comparison, I also looked at the observed tapping overlap in the experimental versus control trials using a Paired *t* test.

Single Larva versus a Larva in a Group. To test the first two predictions (P1, P2) of the tapping hypotheses, I set-up a paired experiment involving 31 larvae from 10 different colonies. Each larva was placed in two situations (order of presentation was alternated between larvae), alone on a branch or within a group of larvae (its original colony), for 5 minutes. To give larvae a period of adjustment after being removed from their original colony, all larvae were individually placed in solitary containers for 5 minutes prior to being tested in the two situations. During the trials, I counted the total number of taps given by the larva, the number of taps within each bout of tapping, and the time at which the first tap occurred. Data were analyzed using the paired *t* test.

Tapping while Foraging. To test P3, I video-recorded the foraging behavior of three colonies during the first hour of feeding. I noted whether larvae were alone on a leaf or with at least one other individual and also whether or not any tapping occurred. A two-sample proportion test was used to compare the proportion of individual (alone) larvae (N = 19) versus grouped larvae of two or more (N = 12) that tapped while feeding on a leaf.

Data for examining P4 came from behavioral observations of group processions described earlier in the Methods section.

Function of Contractions. To examine a potential function of contractions, I tested a set of predictions based on the group coordination hypothesis (contractions serve to initiate or coordinate group movement) (see Table 1). I tallied the observed outcomes of each prediction and compared these scores with those predicted by the coordination hypothesis.

To test the first prediction (P1), that larvae only contract (prior to walking)

when in a group and not when alone, I randomly recorded one walking event for 30 single larvae and scored whether or not it contracted. This was compared with data from larvae in groups. A total of eight to nine larvae were sampled from six colonies and scored for whether or not they contracted before walking; the scores provided a proportion of larvae that contracted per colony. A Mann-Whitney *U* test compared the proportion of single versus grouped larvae that contracted before walking.
Data for predictions P2 and P3 involved preforaging clusters and processionary behavior (see their respective methods sections).

Results

Signals and the Context in which They Occur. The two signals discussed in this paper are tapping and contractions. Tapping is performed by striking the sclerotized anal segment at the tip of the abdomen onto the substrate (Figure 1.2a). It normally occurs in a series of successive taps referred to as a bout of tapping. The average number of taps per bout and bout length (for solitary individuals) are 23.4 ± 5.69, N_{ind} = 20 and 8.34 ± 2.1 s, N_{ind} = 20, respectively, with a mean rate of 2.81 ± 0.21 taps per second, N_{ind} = 20 (see Figure 1.3b). Being a substrate-borne signal produced through a striking motion, the tap is a broad band signal ranging from 0-12 kHz, with frequencies under 6 kHz carrying the most energy (Figure 1.3c).

Tapping is used in a variety of contexts as seen in Table 1.2. Daytime tight clusters of larvae exhibit negligible amounts of signaling aside from the occasional tap or tail flick of individuals. In preforaging clusters, colonies show increased amounts of tapping following the contractions of individuals or the group as a whole. During

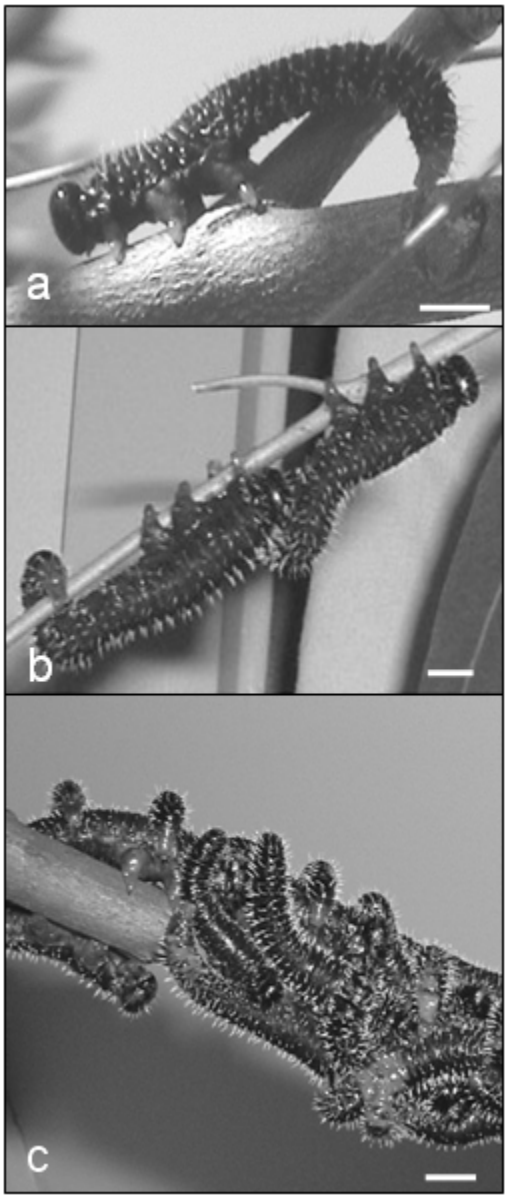
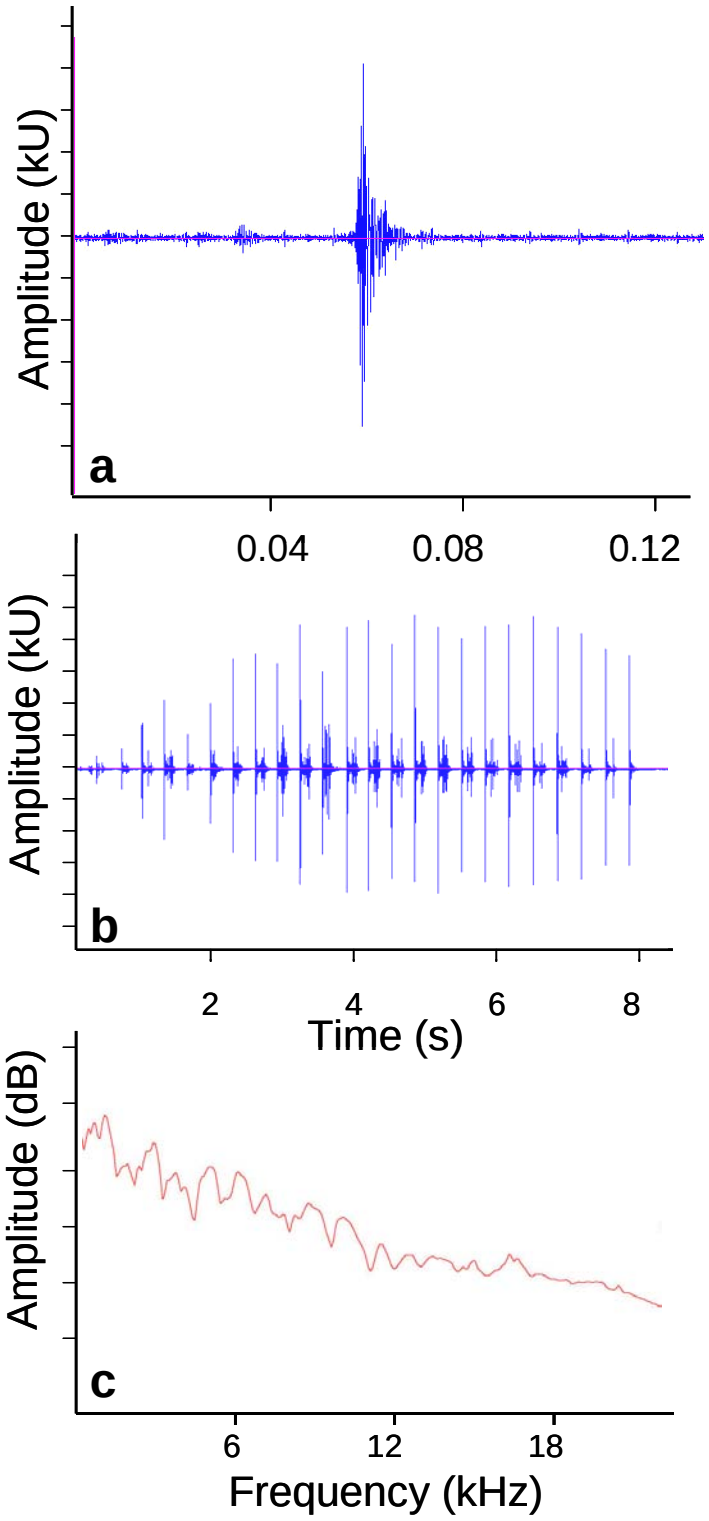


Figure 1.2 a. Late instar *P. affinis* tapping on a branch of *Eucalyptus* sp. Larvae lift their abdomen up to1̣5mm off the substrate and then swing it down to strike the surface with the sclerotized tip of the abdomen. b. Two larvae on a branch demonstrating the typical head-to-tail position of processing larvae. c. Back of a procession line showing the overlapping nature of processions. Several larvae are nestled side by side, maintaining anterior, adjacent, and posterior (with the exception of the rear individuals) contact with other colony members. Scale bars: 5̣mm

Figure 1.3 a. Oscillogram of a single group contraction recorded with the accelerometer at a distance of 3.6cm. b. Oscillogram of a substrate-borne tapping signal by *P. affinis*, recorded with LEEM guitar transducer 12.5cm from the larva. The *y* axis is the amplitude of the signal. c. Power spectrum of the above tapping signal; reveals that it is a broad band signal with energy from 0 to over 12kHz. The *y*-axis is dB (ticks at 20dB intervals), but the measurements are relative, as the recording was not calibrated.


Amplitude (kU)
a
0.04
0.08
0.12
Amplitude (kU)
b
2
4
6
8
Time (s)
Amplitude (dB)
c
6
12
18
Frequency (kHz)

processions, tapping again occurs after contractions although mainly the larvae in back seem to be involved (see Figure 1.4 and procession Results). At the start of foraging, when only one or a few larvae are on a leaf, individuals may tap until several more larvae join the feeding site (this has been noted in *Pergagrapta* sp. as well; (Reid 2004)). Tapping also occurs as colonies reassemble in the early morning after having dispersed to forage.

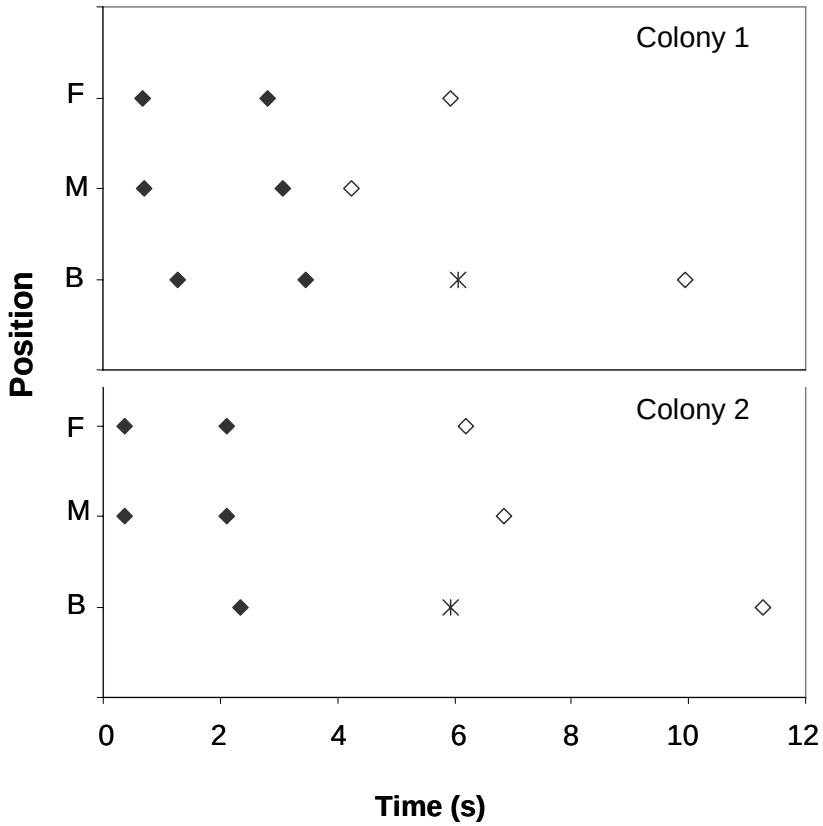


Figure 1.4 Time sequence of signals used by a front, middle and back-positioned larva during a procession event (*filled diamond* contractions, *open diamond* walking, *asterisk* tapping). Contractions occur before walking or tapping and tapping generally occurs in the back of the procession.

Contractions are characterized by a whole-body twitch that appears to be transmitted tactilely as well as through the substrate by the thoracic legs. Figure 1.3a shows an oscillogram of a single group contraction (involving five larvae) recorded by

an accelerometer at a distance of 36 cm. The average duration of a group contraction is 0.135 s ± 0.115 (N = 10, sampled from eight groups of five larvae each), although timing varies according to the synchronicity of the larvae and the size of the group. In general it is an extremely brief signal (on the order of a tenth to a hundredth of a second). It was much harder to get recordings of contractions by individual larvae so no detailed measurements are included; however, visually (via the video recordings) the signal appears to be of a similar duration. As seen in Table 1.2, contractions occur in preforaging clusters and during procession events and, in both cases, contractions precede tapping (see Figure 1.4 for signal timing in procession events). Contractions may be done by single individuals, a subset of the group, or by the colony as a whole (either simultaneously as in preforaging clusters or in a wave of succession as in processions).

Preforaging Clusters. Tight daytime clusters are relatively quiet with little movement or signaling occurring. An increase in signaling (tapping, contractions) by individuals as well as tail movement among the larvae occurs prior to departure. The most conspicuous signal, however, was the group contraction. A group contraction involves the whole colony, where all larvae contract simultaneously. A group will perform one to four of these contractions (mean = 1.72 ± 0.514, N = 51 from four colonies) followed by periods of stillness. The frequency with which group contractions occur increases as the colony nears departure time (Figure 1.5). Colonies (ranging in size from 18-25 larvae) were monitored from 2.5-4 hours prior to departure and in each case showed a dramatic increase in the occurrence of group contractions. This finding provides support for P2, that contractions occur prior to periods of group movement and hence, may facilitate the eventual transition from clustering to processing.

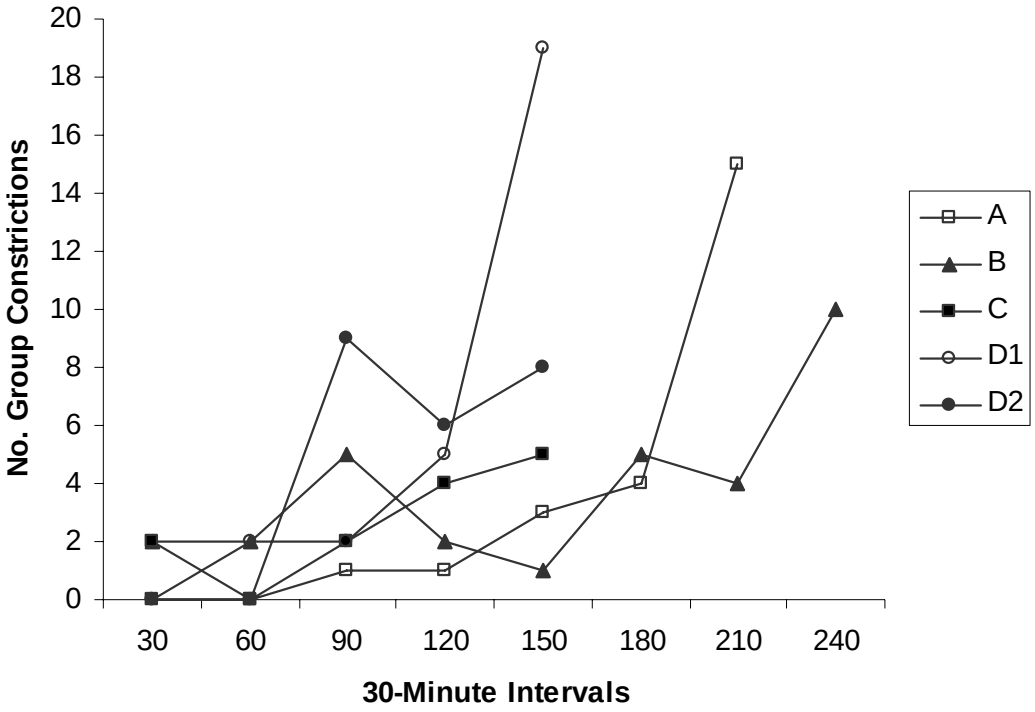


Figure 1.5 Number of group contractions in preforaging clusters recorded at 30-min intervals before a procession. The last point on each line corresponds to the time when the respective colony began to process. *A–D* represent different colonies (ranging in size from 18–25 larvae), with *D1*and *D2* depicting sequences for colony *D* recorded on different days.

Processionary Behavior. Processions of *P. affinis* are marked by periods of movement and stillness. A period of movement begins with one to three waves of contractions (average of 2.75 ± 0.97, $N = 75$) through the group. After the contractions pass through the colony, a subset of the larvae begins tapping and then the group commences its procession along the branch. Eventually the group stops all activity for a period of stillness and then the whole cycle is repeated with the onset of another wave of contractions. Periods of movement during processions last on average for 73.2 ± 32.5 s, $N = 74$ (from seven colonies) while periods of stillness last for an average of 53.7 ± 38.6 s, $N = 67$.

Processions range from being single-file (when on thin branches) to 15-25 individuals wide when on a tree trunk. The width generally depends on the diameter of

the substrate as well as the size of the group (late in the season when several colonies coalesce the width can be upwards of 20 cm). In either case, the larvae remain in physical contact with others by nestling side-to-side and by placing their abdomens on the heads of the larvae behind them (Figure 1.2b,c). The close physical contact appears to aid in the transmission of signals such as contractions, which are both tactile and vibrational in nature and move through the group in a wave-like manner.

It appears as though all members of the group contract when the waves of contractions occur. When I sampled three larvae (one in front, one in the middle, one in the back) in each of 18 procession events, all of them contracted. The contractions generally started in the front and moved to the back (Figure 1.4), as seen by the significantly higher proportion of times that the contractions moved from front to back in each of the six colonies (tested against the null hypothesis of proportion = 0.5) (Wilcoxon signed-rank test: $TS = 21$, $N = 6$, $P = 0.036$). Position in the group therefore affects the timing of contracting in the sequence, but not the actual performance of the signal. This result supports P3 for the function of contractions (see Table 1.1). Contractions are initiated by those directing the movement of the group, since they are significantly more likely to start from the front than from the middle or back of the colony.

For tapping, instead of a positional effect on signal timing, one sees a positional effect on signal use – namely, position affects the likelihood of an individual tapping after the waves of contraction (Figure 1.6; also see Figure 1.4). In each of the six colonies, a significantly higher proportion of the back-positioned larvae tapped than those in the front (Mann-Whitney U test: $W = 25.5$, $N = 6$, $P = 0.0239$). This supports P4 for the function of tapping and is consistent with the group coordination hypothesis (see Table 1). The difference in the proportion that tapped in the back versus the middle or the front versus the middle was not significant. Middle-

positioned larvae generally tapped if the procession became fragmented and stopped signaling once reconnected with the remaining group members.

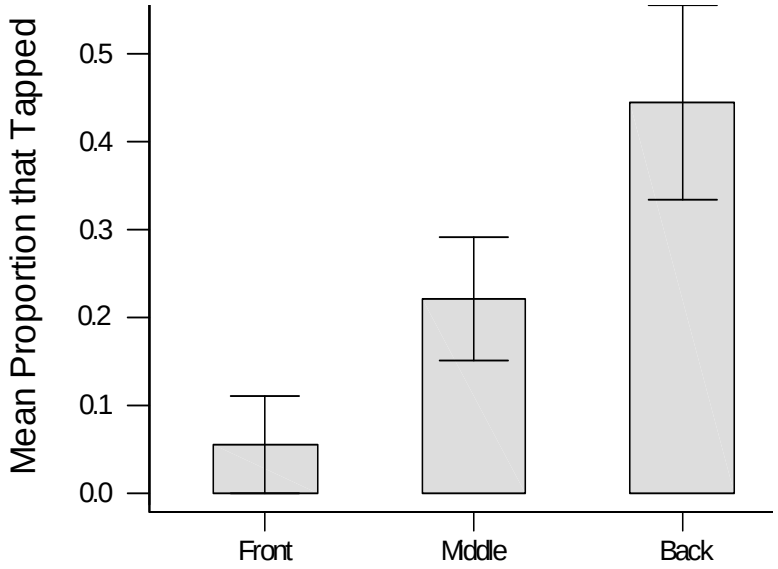


Figure 1.6 Average proportion ± SD (by colony) of front, middle, and back larvae that tapped after contractions during procession events. Those in back tapped significantly more than larvae in the front of the group (Mann–Whitney *U* test: $W = 25.5$, $N = 6$, $P = 0.0239$).

Tapping - A Form of Communication. If tapping is a signal used in communication it should elicit a predictable response in the receiver. Of all the behaviors recorded, the most striking observation was how the tapping of one larva elicited the tapping of the second larva. To quantify this behavior and to test whether or not a larva responds to tapping by signaling itself, I looked at the total time of tapping overlap (when one larva starts tapping and the second individual joins in) in each experimental trial. The observed overlap for each experimental trial was then compared with the observed overlap in the corresponding control trial as well as the expected overlap if it occurred randomly (calculated by the Mathematica™ program, see Methods). The observed tapping overlap in the experimental trials was significantly greater than the overlap in

the control trial (Paired *t* test: $t_{17} = 3.27$, $P = 0.004$, Figure 1.7). Similarly, the observed tapping overlap in the experimental trial was significantly greater than the overlap expected if it occurred randomly (Paired *t* test: $t_{19} = 4.87$, $P = 0.000$). Both results suggest that larvae respond to each other's tapping by signaling and hence provide evidence of communication. The signal may serve as a 'where are you?/I am here' signal for separated larvae. Additionally, the significantly lower tapping overlap in the control versus experimental trials suggests that larvae receive the tapping signal through the substrate as a vibration and not as an air-borne signal.

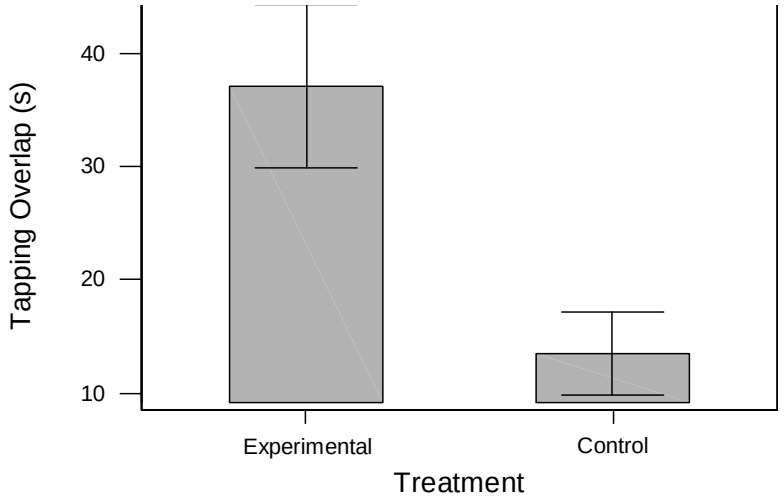


Figure 1.7 Tapping overlap (in seconds) between paired larvae in experimental vs control trials. Significantly more tapping overlap occurred between the two larvae when they were on the same branch (experimental) than when they were on separate branches and could not detect each other (control; paired *t* test: $t_{17} = 3.27$, $P = 0.004$).

Single Larva versus a Larva in a Group. When alone, larvae tapped a significantly greater number of times during the five minute trial than when in a group, with a mean of 98.1 and 38.4 taps, respectively (Paired *t* test: $t_{30} = 5.01$, $P = 0.000$). Single larvae also had a significantly higher number of taps per bout of tapping than when in groups, with a mean of 19.9 and 12.3 respectively (Paired *t* test: $t_{30} = 3.48$, $P = 0.001$).

Both results support P1, which stated that a larva taps more when alone than within a group. Examining the time of the first tap in a trial when larvae were alone versus in a group, showed that lone larvae had a significantly shorter latency to tap than those in a group, with a mean time of 70s versus 136.7s respectively (Paired *t* test: $t_{30} = -3.03$, $P = 0.003$). This supports P2, which stated that larvae would have a shorter latency to tap (i.e. tap sooner) when alone than when in a group. It should be noted that the tapping seen in the group situations mainly occurred because the group started processing after being disturbed to remove and then replace the experimental larva.

Tapping while Foraging. Contrary to P3 for tapping, larvae were significantly more likely to tap on a leaf when alone than when in the presence of at least one other individual (see Figure 1.8; Two-sample proportion test: TS = -3.01, $N_{\text{single larva}} = 19$, $N_{\text{grouped larvae}} = 12$, $p = 0.0011$). In fact, only two of the 12 monitored feeding groups displayed any tapping and both of these were small groups, 2 and 3 larvae each. This result fails to support the competitive signaling hypothesis for tapping and is consistent with the group coordination hypothesis only.

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Results for P4 were discussed in the Procession Behavior segment of the Results section. Larvae demonstrated a positional effect in the use of tapping during processions, with individuals in the back having a higher probability of tapping than those in front. This supports the group coordination hypothesis.

Function of Contractions. Three predictions (P1-P3) were made for the function of contractions and assessed according to their support of the group coordination hypothesis (see Table 1.1). Prediction 1, that larvae only contract (prior to walking) when in a group and not when alone, was tested by comparing the proportion of single larvae versus those in groups that contracted before walking. A significantly higher proportion of the larvae within groups contracted before walking than the lone larvae (Mann-Whitney *U* test: $W = 189$, $N_{group} = 6$, $N_{single} = 30$, $P = 0.000$). This result supports P1 and is consistent with both the group coordination hypothesis.

Results for P2 and P3 were discussed in the Preforaging Cluster and Procession Behavior Results sections, respectively; both supported the group coordination hypothesis.

Discussion

Communication in group-living animals serves many functions ranging from cooperative to competitive purposes. In the case of *P. affinis* larvae, the two signals, contractions and tapping, have a cooperative function. For both signals, the observed results (Table 1) are consistent with the group coordination hypothesis.

Tapping. Tapping occurs in a variety of contexts (Table 2) and no doubt serves a range of functions. Predictions 1-3 (Table 1) dealt with the contexts of single larvae versus groups of larvae and foraging behavior; the observed results were consistent with the group coordination hypothesis only. In these situations the signal appears to function in a 'where are you?' or 'I am here' capacity for separated larvae. A larva taps significantly more and has a shorter latency to tap (i.e., taps sooner) when alone than when in a group. This higher tendency to tap when separated from others would not be expected if it served as a competitive signal; instead one would expect the stimulus of other larvae (and hence competitors) to induce an increase in signaling

behavior.

Similarly, if tapping served as a competitive signal, one would expect the presence of other larvae while foraging on a given leaf (i.e., a limited resource) to invoke the behavior. Although trees have many leaves, they are known to vary in quality (Wheeler and Center 1996; Wheeler 2001), suggesting that a high quality leaf may be a rare find. Additionally, several colonies often inhabit a single tree for up to six months and, depending on larval density, they are capable of a defoliating their host (Carne 1962). Yet, despite the reality of a limited food source, observations indicate that the presence of other larvae *decreased* the likelihood of tapping. The closely related *Pergagrapta* sp. behaves similarly (Reid 2004), with isolated larvae tapping until four to five individuals joined it to forage.

How might the larvae benefit from sharing a leaf with others? In some species, such as the jack pine sawfly (*Neodiprion pratti banksianae*), grouped larvae have a higher probability of establishing a successful feeding site than solitary individuals (Ghent 1960). When feeding on tough plant cuticles like that of pine needles, playing the numbers game has high payoffs, especially for survival rates of first instars (Ghent 1960). An alternative benefit could arise if aggregative feeding induced a change in host plant suitability, making it more palatable, as seen in pipevine swallowtail larvae (*Battus philenor*) and their host *Aristolochia californica* (Fordyce 2003). In this case, the increased suitability is short-lived, making it important to use the resource quickly and hence, further highlighting the benefit of group-feeding. If tapping served as a quality indicator for a given food source, as seen in the stridulation behavior of the striped alder sawfly larvae (Hograefe 1984), perhaps larvae would gain genetic benefits. Since *P. affinis* larvae are thought to live in groups of siblings (at least initially, before colonies merge), they may derive selective advantages from advertising and sharing good resources with colony members. Perhaps tapping is a

way of alerting and guiding other members to a leaf of high quality and larvae on lesser quality leaves do not tap to advertise their food source.

Prediction 4 dealt with tapping in the context of group processions and again the observed results were consistent with the group coordination hypothesis only. A clear positional effect occurred regarding the use of tapping. Individuals in the back of the procession were significantly more likely to tap than those in the front, suggesting that position in the group elicits the behavior. In other processionary larvae, the nudging and jostling of individuals from behind seem to be a cue to encourage hesitant leaders to begin processing (Fitzgerald and Pescador-Rubio 2002; Fitzgerald 2003; Fitzgerald et al. 2004). Similarly, the tapping of rear individuals could be a mechanism for encouraging those in front to start moving. This differs from the 'where are you? or 'I am here' function of tapping seen in context of individuals versus groups or during foraging, but still fits the coordination hypothesis in that it may function to promote group integrity while processing. Animals are known to have variable walking speeds that can cause fragmentation in group movement (Gueron et al. 1996), so perhaps signaling a 'readiness' to move increases the chances of all larvae commencing movement at the same time.

Contractions. Contractions occur in the context of a group environment and are not given by lone larvae (i.e., physically separate), suggesting that their use is stimulated by or dependent upon the presence of other larvae. This supports prediction 1 since the signal is only expected to occur in situations where it may coordinate activity. When in use, larvae typically signal in a synchronized manner involving most, if not all, colony members. A similar phenomenon of a group pulsation was observed in the weevil larvae of *Phelypera distigma*; however, here the signal appears to have a very different function since it only occurs during or after disturbance by a predator (Costa et al. 2004). Larvae of *P. affinis* contract in preforaging clusters and while processing,

but in neither situation are the contractions elicited by an external stimulus. Instead, contractions occur prior to periods of coordinated group movement (supporting P2). Larvae in preforaging clusters contract simultaneously and do so with increasing frequency leading up to the time of procession and departure for foraging. Similarly, contractions occur during processions and move through the entire group in a wave-like manner prior to a bout of walking. In addition to contractions occurring before periods of group movement, we also see an effect of position on the timing of signaling in processions (supporting P3). Larvae in the front of the procession contract before those in the back (Figure 1.4), perhaps as a way of initiating group movement.

Another piece of evidence supporting the group coordination hypothesis involves an important characteristic of the processions – larvae undergo periods of movement and stillness at regular intervals. This pattern of movement and stillness is initiated by the individuals in front and could be a mechanism of maintaining group contact while traveling. Intermittent pauses during processions appear to enable stragglers to catch up with the group; cessation of movement does not occur simultaneously and often those in back continue walking a bit longer before stopping. The pauses could serve other purposes such as providing time for the lead individuals to assess the conditions ahead; however, an obvious result of this pattern is its promotion of group cohesiveness.

Why promote group cohesiveness and coordination? Many organisms live in groups and each is subject to a range of selective pressures pushing it towards this cooperative effort. All aggregating animals such as tadpoles and herds of ungulates have the advantage of a dilution effect when dealing with predators (Watt et al. 1997; Lingle 2001; Spieler 2005). When foraging, groups often increase their foraging efficiency while also increasing the effectiveness of predator vigilance, as seen in

flocks of birds (Boland 2003; Fernandez-Juricic et al. 2004; Dias 2006). And in some instances, the grouping of individuals into a unit creates emergent properties with special functions such as thermoregulation in colonial insects (Simpson 1961; Klingner et al. 2005), aerodynamic lift benefits of formation flying in migrating birds (Lissaman and Shollenberger 1970; Hainsworth 1989), and the creation of spore-bearing structures in slime mold (Parrish and Edelstein-Keshet 1999). Even if individuals vary in the proportion of benefit that they receive (i.e. depending on position in group), they still receive an advantage that is unavailable to the solitary individual.

The larvae of *P. affinis* share many of the benefits of group-living received by other organisms. *P. affinis* has an effective chemical defense in the form of a regurgitant composed of concentrated eucalyptus oils (Morrow et al. 1976) and its potency no doubt increases as the number of larvae employing the strategy increases. Additionally, like the display functions of other organisms such as the giant Thai honeybees (*Apis dorsata*) that shimmer in a wave motion across the colony when a predator approaches (Oldroyd and Wongsiri 2006), larvae rear heads in unison and flick their tails, movements which may make them appear as a large coordinated entity thereby deterring attack. Both of these qualities suggest that predation played a role in shaping the gregarious behavior of the larvae. Aggregation is seen as an effective form of defense in insects, especially when combined with chemical deterrence (Vulinec 1990); however, it is worth noting that no major predators of *P. affinis* are known (Carne 1969). Perhaps, evolutionarily, predators were an important selective pressure but it is likely that other factors, such as the environment, also played a large role. For example, a potential emergent property of gregariousness in these larvae, like other colonial insects, may be enhanced thermoregulation. This could stem from environmental pressure since the sawfly larvae are around during the winter months.

By congregating in clusters during the day they may be able to better absorb solar radiation, thus facilitating physiological processes and growth rates.

Traditionally, the evolution of cooperation through group living has been explained in two ways: 1) kinship theory and 2) reciprocation theory (Axelrod and Hamilton 1981). Many eusocial species such as bees or ants with single-queen colonies exhibit a high degree of relatedness due to their haplodiploid genetic structure and to the fact that all the workers are siblings (Holldobler and Wilson 1990; Boomsma and Ratnieks 1996). A eusocial mammal species, the naked mole rat (*Heterocephalus glaber*), also exhibits high levels of relatedness within colonies by having only one queen and one to three reproductive males at any time (Jarvis et al. 1994). In these systems, individuals gain indirect benefits by cooperating and helping kin. As the group benefits, so does the individual. Larvae of *P. affinis* may reap similar benefits through kinship since the initial colonies are likely composed of siblings. Additionally, it is thought that the main mode of reproduction is amphitokous parthenogenesis (Carne 1962) (although this has not been confirmed genetically). Carne (1962) demonstrated this by dissecting ovaries from newly emerged females and producing normal larvae after a 30-day incubation on sterile agar. If parthenogenesis is common it would give early colonies a relatedness value of one since the siblings would be clones. This level of relatedness, coupled with the overall benefits gained by gregarious living, may help to explain how such a complex signaling system evolved to aid in promoting group integrity. One must note, however, that colonies often merge later in the larval stage so it is unclear how long the kinship benefits would last.

The second condition under which cooperation may occur is through repeated interactions. A broad range of organisms from complex primate societies to group-living birds utilize these tactics to ensure a stable cooperative strategy. In female

baboons (*Papio cynocephalus*), there is a highly significant relationship between grooming equality and the strength of social bonds, indicating the effectiveness of reciprocation in building social ties and hence, cooperation (Silk et al. 2006). Food sharing in juvenile jackdaws (*Corvus monedula*) occurs more frequently between individuals that have shared before (and are reciprocating) as well as between those that have exchanged allopreening sessions (De Kort et al. 2006). In both examples, the cooperation directly stems from repeated interactions where reciprocation is possible.

P. affinis larvae also have repeated contact with each other through their daily rhythm of processing and dispersing to feed and then re-aggregating in a tight group during the day. In this context, cooperation through signaling and coordinated movement benefits all individuals, which may explain why larvae continue to cooperate even when the sibling colonies (with kinship benefits) coalesce with other, unrelated colonies later in the season (this generally starts occurring in the early 3rd or 4th instar, pers. obs). Alternatively, the environment may be sufficiently adverse for solitary or small groups of larvae that cooperation among unrelated individuals arises from by-product mutualism (Mesterton-Gibbons and Dugatkin 1992). Here, the strategy of cooperation stems from 'ordinary selfish behavior' (Eberhard 1975) because defecting (i.e. not signaling or promoting group cohesiveness) could lead to group disintegration, having dire fitness consequences for the individual. In these situations, the benefits of cooperating as a group largely outweigh any costs or conflicts inherent in association.

The adversity of the environment and the corresponding benefits of association are especially pronounced near the end of the larval stage. Larvae descend from their host tree to pupate underground en masse and it is during this time that they have their highest exposure to parasitoids and may suffer high levels of mortality due to

desiccation as they attempt to burrow under the ground (Carne 1966). In fact, the major causes of mortality for *P. affinis* throughout its entire larval and pre-pupal stages are desiccation, parasitism and fungal disease (of water-logged cocoons) (Carne 1969). Being in a group offers the advantage of the dilution effect (from parasitoids) as well as increased chances that at least one individual will successfully break through the ground to burrow. Their cocoons are lined with regurgitant offering chemical protection and a sealant to protect pupae from desiccation (Morrow et al. 1976); both functions of the regurgitant are likely to be enhanced by being in a large group. Therefore, individual larvae gain by cooperating with the group and enhancing its coordination through communication.

Overall, this study confirms the use of acoustic signals for cooperative communication in a gregarious species of sawfly larvae, *P. affinis*. Often when we study group-living organisms, we are quick to assume benefits, and hence, cooperation between the members. However, it is important to consider that, as stated by Parrish et al. (1999), "what appears to be cooperation resulting in cohesion may in fact be conflict veiled by the necessity to minimize the cost of disintegration." Although a group may coordinate its efforts, individual members may still be in conflict suggesting that any signals used in communication have the potential to be competitive or cooperative in nature. Testing between these alternative hypotheses allows us to gain a deeper understanding of not only a specific organism but of the forces that govern cooperation and competition within animal societies. The study of communication is but one of many avenues by which one could determine what is important in balancing the cost and benefit scales in social living.

REFERENCES

Axelrod R, Hamilton WD (1981) The evolution of cooperation. Science 211:1390-1396

Black JM (1988) Preflight signaling in swans - A mechanism for group cohesion and flock formation. Ethology 79:143-157

Boland CRJ (2003) An experimental test of predator detection rates using groups of free-living emus. Ethology 109:209-222

Boomsma JJ, Ratnieks FLW (1996) Paternity in eusocial Hymenoptera. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 351:947-975

Bourne GR (1992) Lekking behavior in the neotropical frog *Ololygon rubra*. Behavioral Ecology and Sociobiology 31:173-180

Bradbury JW, Vehrencamp SL (1998) Principles of Animal Communication. Sinuaer Associates, Inc., Sunderland

Carne P (1962) The characteristics and behaviour of the sawfly *Perga affinis affinis* (Hymenoptera). Australian Journal of Zoology 10:1-34

Carne P (1966) Primitive forms of social behaviour and their significance in the ecology of gregarious insects. Proceedings of the Ecological Society of Australia 1:75-78

Carne P (1969) On the population dynamics of the eucalypt-defoliating sawfly *Perga affinis affinis* Kirby (Hymenoptera). Australian Journal of Zoology 17:113-141

Cocroft RB (2005) Vibrational communication facilitates cooperative foraging in a phloem-feeding insect. Proceedings of the Royal Society of London B-Biological Sciences 272:1023-1029

Cocroft RB, Rodriguez RL (2005) The behavioral ecology of insect vibrational communication. Bioscience 55:323-334

Cokl A, Doberlet MV (2003) Communication with substrate-borne signals in small plant-dwelling insects. Annual Review of Entomology 48:29-50

Costa J, Louque R (2001) Group foraging and trail following behavior of the red-headed pine sawfly *Neodiprion lecontei* (Fitch) (Hymenoptera: Symphyta: Diprionidae). Annals of the Entomological Society of America 94:480-489

Costa JT, Fitzgerald TD, Pescador-Rubio A, Mays J, Janzen DH (2004) Social

behavior of larvae of the Neotropical processionary weevil *Phelypera distigma* (Boheman) (Coleoptera: Curculionidae: Hyperinae). Ethology 110:515-530

Cummings DLD, Gamboa GJ, Harding BJ (1999) Lateral vibrations by social wasps signal larvae to withhold salivary secretions (*Polistes fuscatus*, Hymenoptera: Vespidae). Journal of Insect Behavior 12:465-473

De Kort SR, Emery NJ, Clayton NS (2006) Food sharing in jackdaws, *Corvus monedula*: what, why and with whom? Animal Behaviour 72:297-304

Dias RI (2006) Effects of position and flock size on vigilance and foraging behaviour of the scaled dove *Columbina squammata*. Behavioural Processes 73:248-252

Eberhard MJW (1975) Evolution of social behavior by kin selection. Quarterly Review of Biology 50:1-33

Evans J (1934) Notes on the behavior of the larval communities of *Perga dorsalis* Leach (Hymenoptera, Tenthredinidae). Transactions of the Royal Entomological Society of London 82:455-460

Fernandez-Juricic E, Siller S, Kacelnik A (2004) Flock density, social foraging, and scanning: an experiment with starlings. Behavioral Ecology 15:371-379

Fitzgerald T, Peterson S (1988) Cooperative foraging and communication in caterpillars. BioScience 38:20-25

Fitzgerald TD (1995) The Tent Caterpillars. Comstock Publishing Associates, Ithaca

Fitzgerald TD (2003) Role of trail pheromone in foraging and processionary behavior of pine processionary caterpillars *Thaumetopoea pityocampa*. Journal of Chemical Ecology 29:513-532

Fitzgerald TD, Pescador-Rubio A (2002) The role of tactile and chemical stimuli in the formation and maintenance of the processions of the social caterpillar *Hylesia lineata* (Lepidoptera: Saturniidae). Journal of Insect Behavior 15:659-674

Fitzgerald TD, Pescador-Rubio A, Turna MT, Costa JT (2004) Trail marking and processionary behavior of the larvae of the weevil *Phelypera distigma* (Coleoptera: Curculionidae). Journal of Insect Behavior 17:627-646

Fletcher LE, Yack JE, Fitzgerald TD, Hoy RR (2006) Vibrational communication in the cherry leaf roller caterpillar *Caloptilia serotinella* (Gracillarioidea: Gracillariidae). Journal of Insect Behavior 19:1-18

Fordyce JA (2003) Aggregative feeding of pipevine swallowtail larvae enhances hostplant suitability. Oecologia 135:250-257

Ghent AW (1960) A study of the group-feeing behavior of larvae of the jack pine sawfly, *Neodiprion pratti banksianae* Roh. Behaviour 16:110-147

Gueron S, Levin SA, Rubenstein DI (1996) The dynamics of herds: From individuals to aggregations. Journal of Theoretical Biology 182:85-98

Guerra PA, Mason AC (2005) Male competition and aggregative behaviour are mediated by acoustic cues within a temporally unstructured aggregation. Behaviour 142:71-90

Hainsworth FR (1989) Wing movements and positioning for aerodynamic benefit by Canada geese flying in formation. Canadian Journal of Zoology 67:585-589

Harding BJ, Gamboa GJ (1998) The sequential relationship of body oscillations in the paper wasp, *Polistes fuscatus* (Hymenoptera: Vespidae). Great Lakes Entomologist 31:191-194

Hograefe T (1984) Subtrat-stridulation bei den koloniebildended Blattwespenlarven von *Hemichroa crocea* (Geoff.) (Hymenoptera: Tenthredinidae). Zoologischer Anzeiger 213:234-241

Holldobler B, Wilson EO (1990) The Ants. Harvard University Press, Cambridge

Janik VM, Slater PJ (1998) Context-specific use suggests that bottlenose dolphin signature whistles are cohesion calls. Animal Behaviour 56:829-838

Jarvis JUM, Oriain MJ, Bennett NC, Sherman PW (1994) Mammalian eusociality - A family affair. Trends In Ecology & Evolution 9:47-51

Jolivet P, Vasconcellos-Neto J, Weinstein P (1990) Cycloalexy: a new concept in the larval defence of insects. Insecta Mundi 4:133-142

Klingner R, Richter K, Schmolz E, Keller B (2005) The role of moisture in the nest thermoregulation of social wasps. Naturwissenschaften 92:427-430

Langbauer WR (2000) Elephant communication. Zoo Biology 19:425-445

Lingle S (2001) Anti-predator strategies and grouping patterns in white-tailed deer and mule deer. Ethology 107:295-314

Lissaman PB, Shollenberger C (1970) Formation flight of birds. Science 168:1003-1005

Mesterton-Gibbons M, Dugatkin LA (1992) Cooperation among unrelated individuals - Evolutionary factors. Quarterly Review of Biology 67:267-281

Michelsen A, Flemming F, Gogala M, Traue D (1982) Plants as transmission channels for insect vibrational songs. Behavioral Ecology and Sociobiology 11:269-281

Moller P (1976) Electric signals and schooling behavior in a weakly electric fish, *Marcusenius cyprinoides* L (Mormyriformes). Science 193:697-699

Morrow P, Bellas T, Eisner T (1976) Eucalyptus oils in the defensive oral discharge of Australian sawfly larvae (Hymenoptera: Pergidae). Oecologia 24:193-206

Nelson MC, Fraser J (1980) Sound production in the cockroach, *Gromphadorhina portentosa* - Evidence for communication by hissing. Behavioral Ecology and Sociobiology 6:305-314

Oldroyd BP, Wongsiri S (2006) Asian honey bees: Biology, conservation and human interactions. Harvard University Press, Cambridge

Parrish JK, Edelstein-Keshet L (1999) Complexity, pattern, and evolutionary trade-offs in animal aggregation. Science 284:99-101

Radford AN (2004a) Vocal coordination of group movement by green woodhoopoes (*Phoeniculus purpureus*). Ethology 110:11-20

Radford AN (2004b) Vocal mediation of foraging competition in the cooperatively breeding green woodhoopoe (*Phoeniculus purpureus*). Behavioral Ecology and Sociobiology 56:279-285

Reeve HK (1991) Polistes. In: Ross KG, Matthews RW (eds) The Social Biology of Wasps. Comstock Publishing Associates, Ithaca, pp 99-148

Reid SF (2004) Group foraging, navigation and morphology of the sawfly larvae *Pergagrapta* (Hymenoptera: Symphyta: Pergidae). In: School of Botany and Zoology. University of Australia, Canberra, pp 56

Ruf C, Costa JT, Fiedler K (2001) Trail-based communication in social caterpillars of *Eriogaster lanestris* (Lepidoptera: Lasiocampidae). Journal of Insect Behavior 14:231-245

Russ K (1969) Beiträge zum Territorialverhalten der Raupen des Springwurmwicklers, *Sparganothis pilleriana* Schiff (Lepidoptera: Tortricidae). Pflanzenschutz Ber. Wein. 40:1-9

Savoyard JL, Gamboa GJ, Cummings DLD, Foster RL (1998) The communicative meaning of body oscillations in the social wasp, *Polistes fuscatus* (Hymenoptera, Vespidae). Insectes Sociaux 45:215-230

Silk JB, Alberts SC, Altmann J (2006) Social relationships among adult female baboons (*Papio cynocephalus*) II. Variation in the quality and stability of

social bonds. Behavioral Ecology and Sociobiology 61:197-204

Simpson J (1961) Nest climate regulation in honey bee colonies - Honey bees control their domestic environment by methods based on their habit of clustering together. Science 133:1327-1333

Spieler M (2005) Can aggregation behaviour of *Phrynomantis microps* tadpoles reduce predation risk? Herpetological Journal 15:153-157

Stein AC, Uy JAC (2006) Plumage brightness predicts male mating success in the lekking golden-collared manakin, *Manacus vitellinus*. Behavioral Ecology 17:41-47

Tibbetts EA (2002) Visual signals of individual identity in the wasp *Polistes fuscatus*. Proceedings of the Royal Society of London Series B-Biological Sciences 269:1423-1428

Vulinec K (1990) Collective security: Aggregation by insects in defense. In: Evans L, Schmidt J (eds) Insect Defenses. State University of New York Press, Albany, pp 251-288

Watt PJ, Nottingham SF, Young S (1997) Toad tadpole aggregation behaviour: evidence for a predator avoidance function. Animal Behaviour 54:865-872

Wheeler GS (2001) Host plant quality factors that influence the growth and development of *Oxyops vitiosa*, a biological control agent of *Melaleuca quinquenervia*. Biological Control 22:256-264

Wheeler GS, Center TD (1996) The influence of Hydrilla leaf quality on larval growth and development of the biological control agent *Hydrellia pakistanae* (Diptera: Ephydridae). Biological Control 7:1-9

Yack J, Smith M, Weatherhead P (2001) Caterpillar talk: Acoustically mediated territoriality in larval Lepidoptera. PNAS 98:11371-11375

CHAPTER 2

COOPERATIVE SIGNALING AS A POTENTIAL MECHANISM FOR COHESION IN A GREGARIOUS SAWFLY LARVA, *PERGA AFFINIS*[*]

Abstract

During periods of travel or dispersive activities (e.g. foraging), group-living animals face the common challenge of maintaining a cohesive unit. At the basic level, this challenge is no different for vertebrates than it is for arthropods and is solved through communication. Gregarious larvae of the Australian sawfly, *Perga affinis*, communicate via vibrational signals. The most common signal, tapping, involves striking the substrate with the sclerotized tip of the abdomen. This study investigates the role of tapping as a mechanism of cohesion, specifically in situations between a separated larva and a group. As nomadic foragers that daily move to new feeding locations and readily coalesce with other colonies, the possibility of separation and potential re-aggregation arises regularly. Experiments demonstrated that tapping facilitates cohesion as groups responded to the tapping of lone larvae and did so preferentially over other larval behaviors. Additionally, separated larvae respond to tapping by the group through increased walking activity. It is also possible that they receive directional information from the group's vibratory signals although visual cues may influence orientation as well. Tapping represents a cooperative signal and, as such, I investigated the level of investment of both parties in the communicative exchange. While individual larvae invested more in the exchange than the group, the exchange is analogous to the Raise-the-Stakes model of cooperation in that groups gradually increased their investment according to the cumulative time spent tapping by the lone larva. The mutual but asymmetrical benefits received through cooperation

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are discussed and compared with similar situations between parents and offspring. Not all larvae in the group participated equally, suggesting individual differences in signaling propensity or strategy.

Introduction

Although plants live in groups, the terms gregarious and group-living generally apply to the animal world, and particularly to those that are mobile. By definition, groups are composed of separate entities and it is the mobility of these entities that gives them the option of forming groups or remaining apart. Because they have both capacities, groups require active mechanisms for maintaining their cohesive structure. Communication provides such a mechanism and is essential to the coordination and cohesion of social groups at all levels of biological organization. Through chemical signaling, individual protists aggregate to form slime molds, slug-like structures that move and forage in a coordinated manner (Raper 1984). Similarly, other organisms use signals such as the pheromone trails of processionary caterpillars (Fitzgerald and Peterson 1988) and the acoustic contact calls of foraging primates, birds, and social carnivores (Boinski 2000; Greenberg 2000; Holekamp et al. 2000) to communicate with group members and facilitate cohesion during periods of movement.

Larvae of the Australian sawfly, *Perga affinis*, live in colonies and, as nomadic foragers move to new feeding and resting sites on a daily basis. Such regular periods of movement, dispersion (for feeding), and re-aggregation present the constant challenge of preventing fragmentation and separation. The fact that colonies not only remain intact but readily coalesce with other groups to form large masses of individuals suggests they have an effective mechanism for maintaining group integrity. *P. affinis* larvae communicate via two known vibrational signals, contractions and tapping, both of which are cooperative in function (Fletcher 2007).

Contractions (a whole-body twitch) are given by all group members with increasing frequency before the colony departs to forage and may function to coordinate their processing and foraging activity. Tapping involves striking the substrate with the sclerotized tip of the abdomen and has been shown to elicit tapping in another larva, suggesting a call and response type function (Fletcher 2007). Here, I specifically look at tapping to determine if it is used to promote group cohesion in pergid colonies by serving as a reciprocal 'where are you?/we are here' signal between groups and separated larvae.

Communication that enhances cohesion is a type of cooperative signaling. Cooperative signaling implies the receipt of benefits by both the senders and receivers, be they direct or indirect in nature. Although both parties gain in an exchange, the relative amount may vary and this, in turn, may affect the investment level of the constituents. Contact calls are a cooperative signal given by members in a group when foraging or traveling, which enable them to stay connected acoustically despite physical barriers and/or visual impairment (i.e. primates and birds foraging in dense forest, (Boinski and Mitchell 1992; Greenberg 2000). Such signals benefit all members and hence one would expect relatively equal participation. Other signals, such as those for re-uniting individuals after separation, are also cooperative in function but the benefits received by members may vary according to the circumstances. For example, communicating to facilitate a reunion between parents and offspring after separation offers mutual but asymmetrical benefits to the two parties. Although both individuals share genetic interests, the fitness consequences of young not uniting with their parents are much greater than they are for the parents (if/when the parent has multiple offspring or breeding seasons) (Trivers 1985; Insley 2001). Such cases predict asymmetrical investment in the signaling and re-uniting process.

When examining tapping as a mechanism of cohesion between groups and separated larvae in *P. affinis*, I also look at investment levels in this cooperative signaling exchange. Given that a separated individual is alone suggests that it has more to gain by joining a group than the group does in receiving an additional member. If true, one would expect a higher investment in signaling by the separated larva than the corresponding group. To test both the potential cohesive function of tapping and relative investment levels of the two parties in the cooperative exchange, I have formulated four predictions (Ps); the first two deal directly with cohesion while the second two relate to the investment levels. If tapping serves as a mechanism for cohesion between groups and separated larvae, I predict that: (P1), groups respond to the tapping of a lone larva by signaling and thereby provide a stimulus for the larva; and (P2), there is evidence of directional information acquired through the signal (i.e. the larva walks towards the group). A variety of organisms use acoustic signals that both elicit a response and provide directional information to the recipient as seen in adult murrelets (*Synthliboramphus antiquus*) vocalizing with their young (Jones et al. 1987), mating duets in spiders and insects (Rovner and Barth 1981; Ota and Cokl 1991), and vibrational distress calls of trapped worker ants (Markl 1965).

In relation to the investment in signaling, I predict (P3), that the single larva invests more in the communication exchange than the group, and (P4), that the group's response or investment level is influenced by the single larva's behavior. Each prediction stems from the assumption of mutual but asymmetrical benefits in the communication exchange and the tests offer insight into the type of cooperation taking place. Both interdependent (Roberts 2005) and reciprocal cooperation through the Raise-the Stakes strategy (Roberts and Sherratt 1998) will be discussed.

Methods

Daily patterns of *P. affinis*. Newly emerged larvae remain in sibling groups (20-30 individuals) forming tight cycloaxelic circles during the day (a rosette pattern with heads pointed outward and the posterior abdomens pointed inward, see (Jolivet et al. 1990). At night, they disperse to the leaf's edge to feed, returning again to their clusters in the morning. This nocturnal activity pattern continues throughout the 5-6 instars of the larval stage but as they grow, they shift their day time clusters from leaves, to the tree branches and occasionally the trunk. Much of the acoustic signaling occurs during the daily patterns of movement: processions to foraging sites and then the re-aggregation of clusters at dawn. The experimental work presented here focuses on the use of tapping when individuals become separated from the group. This situation occurs regularly both during processions and the morning re-aggregations.

Group versus Single Larva Experiment. The experiment was designed to test the effect of signaling (i.e. tapping) by a single larva on the behavior of a group of larvae. The overall hypothesis that tapping serves as a mechanism for promoting group cohesion leads to a series of 4 predictions. I will first discuss the overall experiment and then detail the specific tests for each prediction.

I conducted a paired experiment with 12 control and 12 experimental trials. Each group consisted of 4-5 individuals. This is within the natural range of group size (albeit, the low end) and was chosen so that I could individually mark and record behavior of each larva. In the experimental trials, a group of larvae and a single larva resided on the same branch but were separated by a card board divider (each sat at a distance of 10cm from the divider). This allowed the transfer of vibrational information between the two sides while excluding any possible visual cues of conspecifics. Trials lasted for 20 minutes and were recorded with a Sony Digital 8 DCR-TRV830 camcorder. Additionally, a phonocatridge placed onto the substrate

and attached to a Sound Devices MP-1 preamplifier fed into the camcorder thereby allowing simultaneous acoustic and visual information to be recorded. The control trials were similar except that the single larva and the group sat on different branches, separated by a distance of 45cm (Figure 2.1).

Larvae were placed in their experimental groups at least 24 hours prior to the testing. Due to the difficulty in getting the groups of larvae to cluster for the experiment, all control trials occurred before the experimental trials. In this way an accurate baseline of group signaling activity could be assessed before the influence of a single larva was added.

Data collection on the single larva from the videotapes involved recording the number of taps as well as the duration of the following behaviors; tapping, walking (towards or away from the group), and searching (lifting the head and 1st pair of thoracic legs off the substrate). Larvae in the groups were individually marked, allowing me to collect behavioral data on them separately. The behaviors recorded for the group were slightly different as they include contractions (a whole-body twitch), which only occurs in group settings (Fletcher 2007). For signaling, I recorded the number and timing of contractions, group contractions (given by group simultaneously), and tapping. The only other behavior monitored was walking (groups did not show the searching behavior).

Test of Prediction 1. If tapping acts as a call and response mechanism to facilitate cohesion, the first prediction, (P1), is that the group will respond to the tapping of a separated larva by signaling. To test this, I looked at each behavior performed by the single larva and noted whether or not the group responded (a response was any signaling behavior, i.e. contraction, group contractions (GC), or tapping, that occurred within 10 seconds of the single larva's tapping). The response of the group was examined in both control and experimental trials, thus providing a baseline of

signaling overlap (within 10sec of the single larva's tapping) between the single larva and the group when no vibratory cues were available. I conducted a binary logistic regression with the group response as the binary dependent variable. The predictors were trial number (to control for repeated measures per trial) and the single larva's behavior. A separate analysis also included a predictor variable for control vs experimental trials to verify that the group's response behavior differed between the two set-ups.

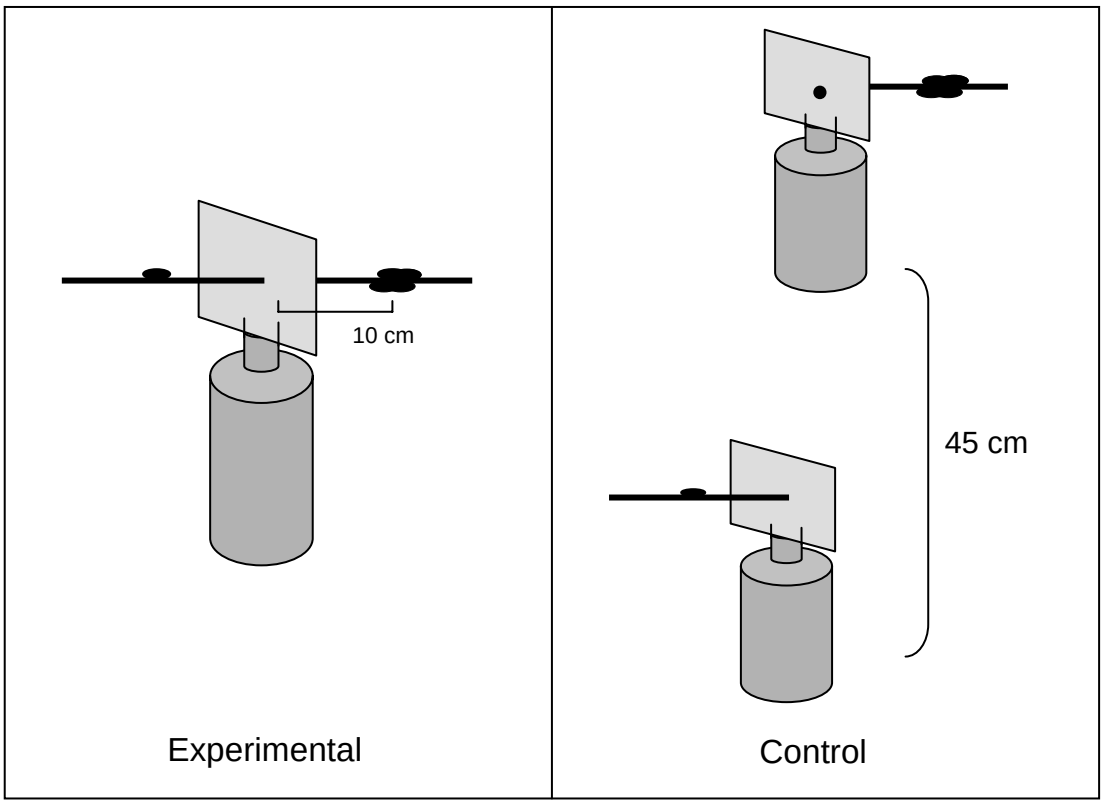


Figure 2.1 Experimental and control set-ups for the experiment. Both the group and the single larva were 10 cm from the center divider at the start of the trial.

Test of Prediction 2. The second prediction, (P2), for tapping to serve as a mechanism of cohesion is that tapping provides directional information to the receiver. This would be best demonstrated by the larva walking towards the group. To test this,

I quantified the total amount of time spent walking by the single larva as well as the total time spent walking towards the center (which is towards the group in experimental trials) in both control and experimental trials. I then examined the proportion of time in which the single larvae walked towards the center in both trial types. These comparisons allowed for direct examination of differences in larval walking behavior between the two test situations. Data were analyzed using the Wilcoxon signed rank test and compared against the null hypothesis that larvae spent an equal total time walking and an equal proportion of time walking towards the center in experimental vs control conditions.

Tests of Prediction 3. The third prediction, (P3), states that the single larva invests more in the exchange than the group. To test P3, I looked at three different measures of investment in communication for the group vs the single larva: (i) who initiates behavioral activity and signaling during the experimental trials, (ii) the per capita number of taps given during the trial, and (iii) the rate of tapping (average number of taps/bout of tapping in the trial). Note: taps are given in bouts, which are continuous repeats of individual taps; typically there are 17-29 taps/bout.

To examine the first measure of investment, who initiates behavioral activity, I calculated a ratio for both the individual and the group of the number of times they initiated a behavior (1^{st} to behave with no prior stimulus) / the number of times they behaved 2^{nd} in a sequence (within 10 seconds of the other's activity). A ratio of one indicates an equal likelihood of initiating and responding whereas a ratio greater than one suggests a propensity to initiate and less than one, a propensity to respond. A ratio was calculated for each single larva and group in the experimental trials and compared using a Wilcoxon's signed rank test.

As a second comparison for who initiates activity, I specifically looked at signal exchanges (a signal exchange occurs when either the individual or group signals

and the other responds by signaling within 10 seconds). The first comparison involved any behavioral activity, whereas, this second one involved only signals (tapping for the individual larva and group contractions or tapping for the group). For each experimental trial, I calculated the proportion of times the single larva initiated the exchanges. I tested this proportion against the null median of 0.5 using the Wilcoxon signed rank test.

The second measure of investment examined the per capita number of taps given during the experimental trial. For the single larva, this was just the total number of taps in the trial. For the group, I calculated the total number of taps and divided it by the number of individuals that tapped during the trial, giving me the per capita number of taps for the larvae involved. A Wilcoxon signed rank test compared these two values for each trial.

The third measure of investment compared the tapping rate for the single larva and larvae in the group using a general linear model. The dependent variable was rate of tapping and the predictors were trial number (to control for repeated observations), who initiated the signaling (Ind or Grp), who did the signaling (Ind/Grp), and time in trial (to see if rate changed over the course of the 20-min trial).

Tests for Prediction 4. Prediction 4, (P4), states that the group's response (i.e. magnitude) is influenced by the single larva's behavior (i.e. signaling activity). I tested this prediction in two ways. The first used a Pearson's correlation test to compare the total number of taps given by the group with the total number of taps given by the single larva in a trial. The second involved examining the timing (the first appearance) of specific behaviors by the group as a function of the cumulative time spent tapping by the single larva. The three group behaviors of interest were the first time that: 1) two or more larvae tapped, 2) a group contraction occurred and 3) one or more larvae started walking. Each of these behaviors may indicate an increase

in the level of investment from the group; tapping of two or more individuals may be less of a 'commitment' than having all individuals participate in a group contraction, which again may be less than having at least one individual begin walking (suggesting that the group may move). If the above list represents an order that ranks the group's investment, one would expect that a higher threshold of cumulative tapping would be needed from the single larva before the group would show each consecutive behavior. A Cox regression was used to analyze the data. This method does not assume a particular distribution and it models time-to-event data in the presence of censored cases (i.e. trials where the group did not perform one of the behaviors being measured) (Kalbfleisch and Prentice 2002).

Participation of Individuals within the Group. To examine the distribution of signaling effort within the group, I counted the total number of taps given by the group as a whole as well as the total given by each individual. I compared the number of taps given by the individuals with the number expected if the signaling were divided equally for each group using a Chi-square test.

Results

Test for Prediction 1 – Responding to Signals. Individual larvae performed several behaviors during the course of a trial, four of which were quantified: tapping, walking towards the center, walking away from the center and searching (lifting the head and 1st pair of thoracic legs off the substrate). The odds of a group responding to the activity of a larva were significantly affected by the specific behavior employed. The group had significantly higher odds of responding to tapping than any other behavior, providing strong support for P1 (Binary logistic regression, Wald = 46.516, df = 3, p = 0.000; see Figure 2.2). There was no significant difference in the odds of responding to the larva walking towards vs away from the center divider; however, pairwise tests

indicated that the group was more likely to respond to walking (towards the center WC; towards the edge WE) than to the searching behavior (WC: Wald = 9.954, df = 1, p = 0.002; WE: Wald = 5.044, df = 1, p = 0.025).

A separate model with the predictor variable for control vs experimental trials indicated a highly significant difference in the odds of the group responding (or behaving at all) between the two trial types (Binary logistic regression, Wald=84.053, df=1, p=0.000). Indeed, at least half of the groups showed no activity during the entire control trial; this again provides support for P1 since the observed activity mainly occurs in the presence of a separated signaling larva.

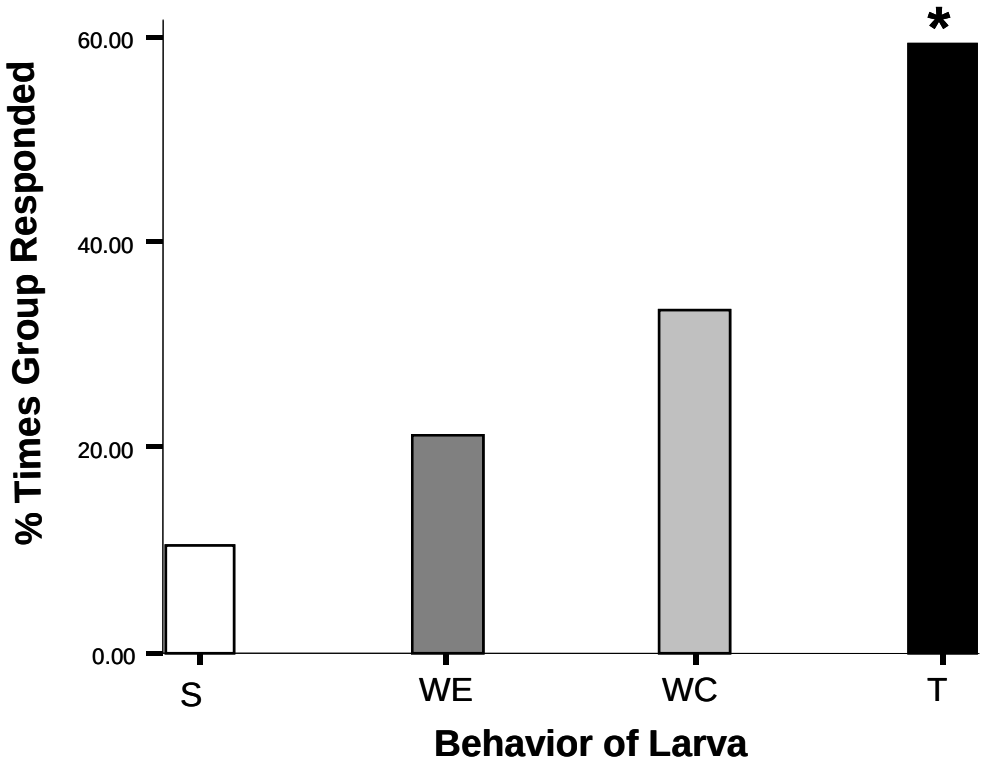


Figure 2.2 Percentage of times groups signaled in response to the different behaviors of the single larva. The odds of a group responding were significantly higher for tapping than any of the other behaviors performed by the single larva (T – tapping, WC – walking towards center, WE – walking towards edge, S – searching; Binary logistic regression, Wald for behavior = 46.516, df = 3, p = 0.000).

Tests for Prediction 2 – Evidence of Directional Information. To look for evidence of directional information, I examined the walking behavior of the single larva under experimental and control conditions. Larvae spent a higher proportion of time walking towards the center (towards the group) in experimental than control trials, suggesting the receipt of directional information (median of 0.63 and 0.41, respectively; Wilcoxon signed rank test, W=47, N=10, p=0.053; Figure 2.3). Larvae also spent a significantly greater amount of time walking in experimental vs control trials (median of 130s and 49.5s, respectively; Wilcoxon signed rank test, W=52, N=10, p=0.014), indicating an overall higher activity level in the presence of signaling.

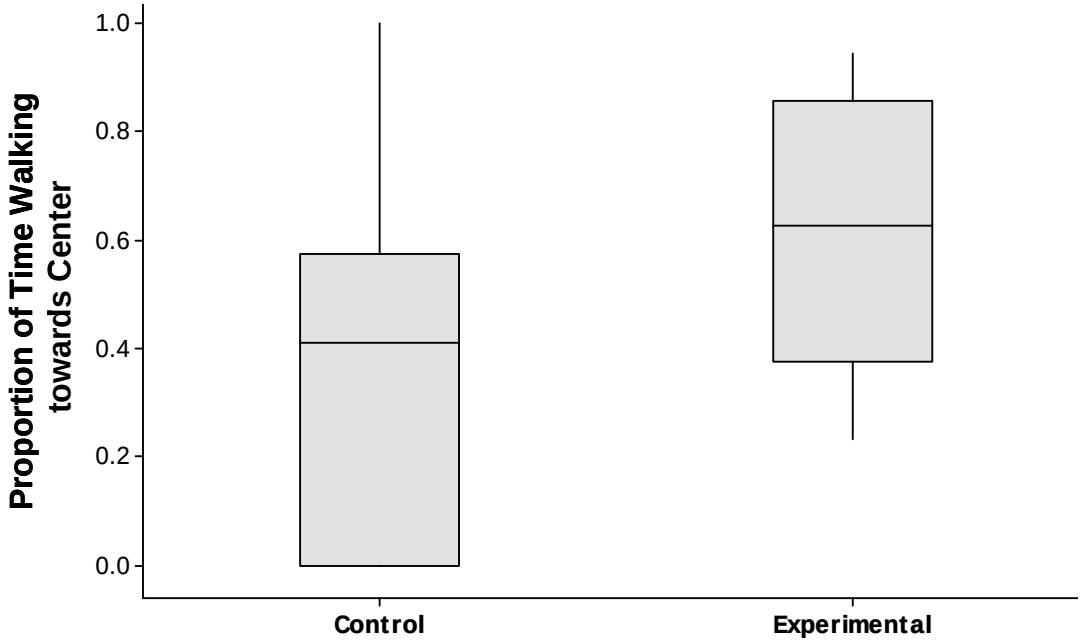


Figure 2.3 Median proportion of time spent walking towards the center during control and experimental trials. Larvae spent a higher proportion of time walking towards the center when receiving signaling information during the experimental trials than during the 'quiet' control trials. Wilcoxon signed rank test, W=47, N=10, P=0.053.

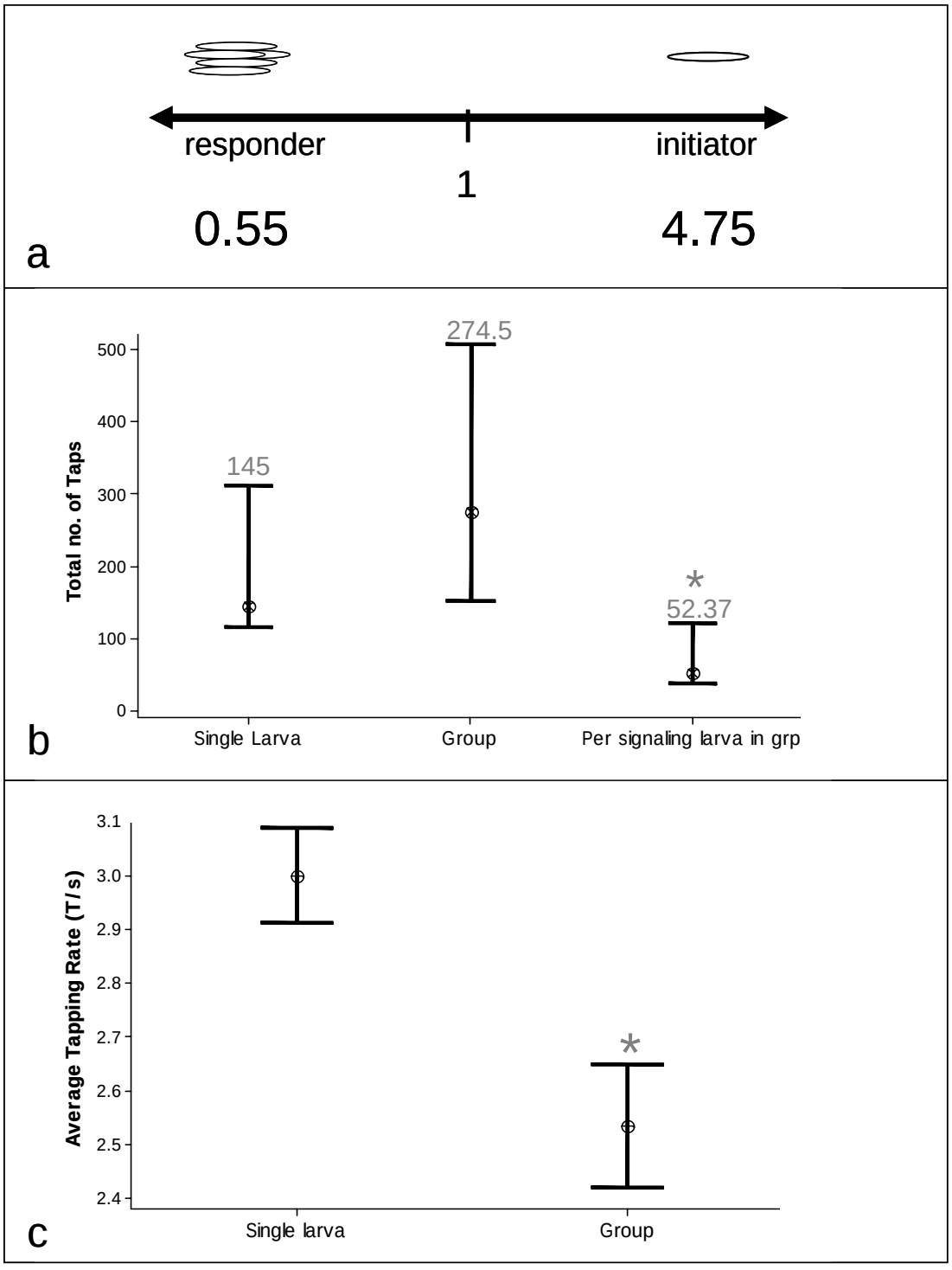
Tests for Prediction 3 – Investment in Exchange. The three measures of investment examined were (i) who initiated behavioral activity and signal exchanges, (ii) per capita number of taps given in a trial and (iii) rate of tapping. The single larvae demonstrated a strong propensity to initiate activity with a median initiator ratio 4.75 while groups had a significantly lower median ratio of 0.55, indicating a higher tendency to respond (Wilcoxon signed rank test, N=11, Z=-2.934, p=0.003; Figure 2.4a). Likewise, when examining signal exchanges, the single larvae initiated a significantly higher percentage of the exchanges than the group with a median of 93% (Wilcoxon signed rank test, N=10, W=55, p=0.006). Both measures demonstrate a higher propensity for the single larvae to initiate activity and signaling.

The median number of taps given by the single larvae versus the groups as a whole was 145 and 274.5, respectively, which was not significantly different in a pairwise comparison (Wilcoxon signed rank, N=12, Z=-0.941, p=0.347; Figure 2.4b). When looking at the per capita investment in tapping, however, the situation changes; single larvae tap significantly more than individual larvae within a group (median of 146 and 52.37 taps, respectively, Figure 2.4b; Wilcoxon signed rank test, N=12, Z=-2.981, p=0.003). Therefore, the per capita investment in tapping is greater for the single larvae.

When examining the rate of tapping for groups versus single larvae, I used a general linear model, which allowed me to control for trial number, who initiated the signaling, and the time that the tapping took place in the trial. Of all the predictors tested, only identity of the signaler (group or single larva) affected tapping rate. Single larvae tapped at a significantly higher rate than larvae in groups (Figure 2.4c; GLM, $F_{1,203} = 32.08$, $p < 0.0001$).

All three measures of investment indicate that the single larvae invest more in the communication exchange than the group, thereby providing strong support for P3.

Figure 2.4 Measures of investment in communication exchange. a. Diagram of the initiating activity ratio for groups versus single larvae. A value of 1 indicates an equal propensity to initiate and respond while greater than 1 demonstrates a higher propensity to initiate and less than 1 suggests a higher propensity to respond. Single larvae had significantly higher ratios than the corresponding groups (Wilcoxon signed rank test, N=11, Z=-2.934, p=0.003) b. Total number of taps given during the 20 minute trial. Circular mark indicates median and interval bars are represent 2 SD. Single larvae tapped significantly more than individual larvae in the group (Wilcoxon signed rank test, N=12, Z=-2.981, p=0.003) c. Average rate of tapping (± SD) in trials for single vs grouped larvae. Identity of the tapper was the only significant predictor in the GLM where single larvae tapped at a significantly higher rate than larvae in groups (GLM, $F_{1,203} = 32.08$, $p < 0.0001$).


responder
initiator
1
0.55
4.75
a
Total no. of Taps
500
400
300
200
100
0
145
274.5
*
52.37
Single Larva
Group
Per signaling larva in grp
b
Average Tapping Rate (T/s)
3.1
3.0
2.9
2.8
2.7
2.6
2.5
2.4
*
Single larva
Group
c

Tests for Prediction 4 – Group Response Influenced by Individual's Behavior.

To determine whether the magnitude of a group's response was influenced by the signaling behavior of the single larva, I first compared the total taps given by groups with the total taps given by the single individuals using a Pearson's correlation test. There is a positive, significant correlation between these two factors, suggesting that the more a single larva taps, the greater response it will receive from the group (Figure 2.5; Pearson's correlation = 0.697, N=11, p=0.017).

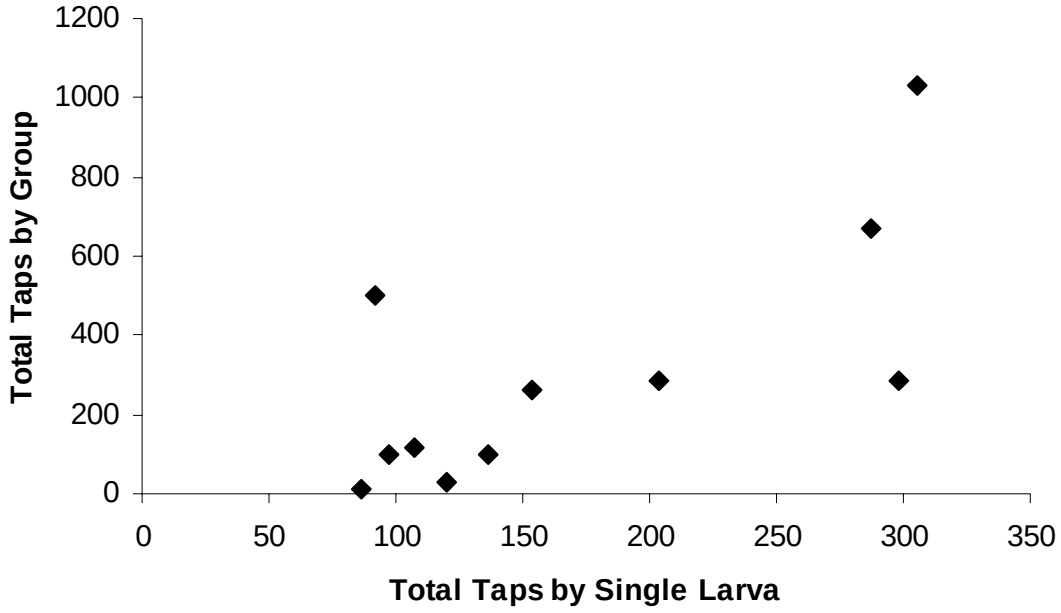


Figure 2.5 Correlation between the signaling input of the single larva and the group's response, measuring total number of taps during the trial (Pearson's correlation = 0.697, N=11, p=0.017).

As another test of this prediction, I looked at how the cumulative time spent tapping by the single larva affected the timing (or first appearance) of three different behaviors by the group, where each consecutive behavior may indicate a higher investment in the group's response. The three behaviors were the first time that two or more individuals tapped, the first group contraction, and the first time one or more

larvae walked. Results from the Cox regression showed a significant difference between the timing of the three behaviors (Cox regression, $\chi^2 = 43.464$, df=12, p=0.000). Pairwise comparisons indicated that tapping of 2 or more larvae occurred significantly earlier (i.e. required less cumulative tapping time to elicit the behavior) than group contractions, which occurred significantly earlier than walking. Figure 2.6 demonstrates this graphically by showing the cumulative failure curves for each behavior ('failure' is the term used to describe the occurrence of the specified event). The lines are distinct, suggesting that each behavior has a different 'threshold' of signaling required before it is elicited.

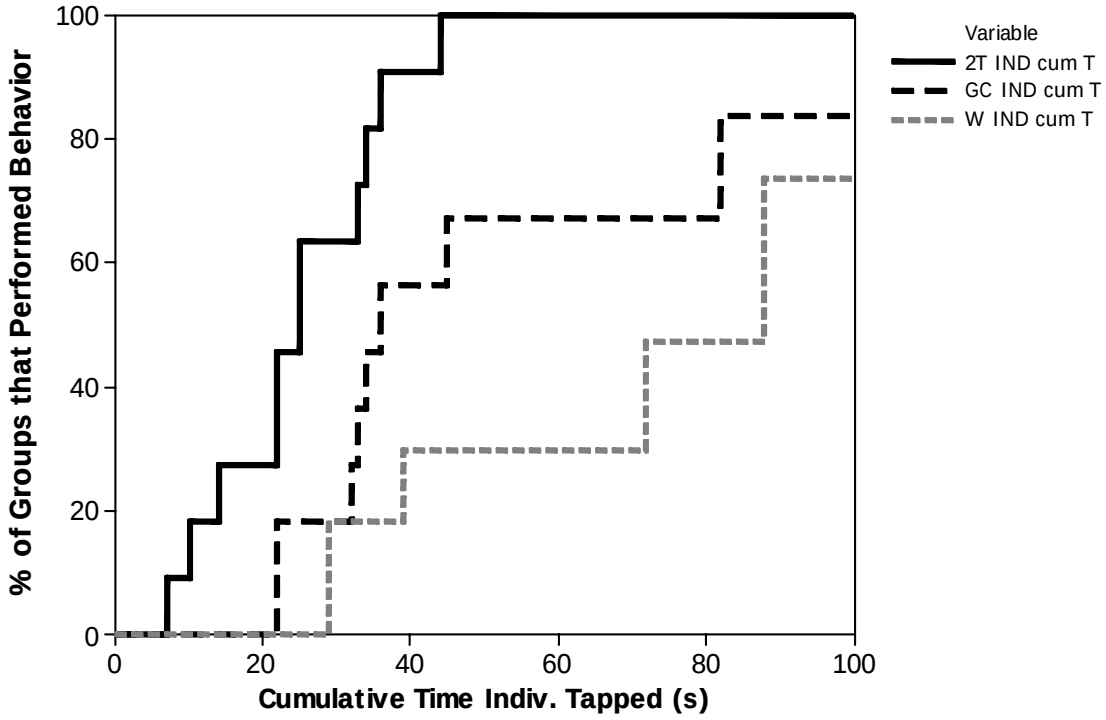


Figure 2.6 Cumulative failure curves for the first occurrence of three different behaviors in groups (solid line - 2+ larvae tapping, medium dashed line - a group contraction (GC), and small dashed line - walking (W)). The percentage of groups that first demonstrated each behavior is plotted as a function of the cumulative time spent tapping by the single larva. The curves are significantly different, indicating a lower 'threshold' to elicit tapping than GC and a lower threshold for GC than W (Cox regression, $\chi^2 = 43.464$, df=12, p=0.000).

Participation of Individuals within the Group. Larvae within groups did not participate equally in the signaling activity. A chi-square test performed on 9 of the groups (the other 3 had individuals that did not tap and could not be included) showed that the distribution of tapping was significantly different from that expected if shared equally (Table 2.1).

Table 2.1 Chi-square Tests on Tapping Distribution in Groups

Group	χ^2	df	P-value
A	41.69	3	< 0.0001
B	151.25	4	< 0.0001
C	96.03	3	< 0.0001
D	67	3	< 0.0001
E	131.83	4	< 0.0001
F	128.71	5	< 0.0001
G	270.76	3	< 0.0001
H	153.56	4	< 0.0001
I	23.71	3	< 0.0001

Discussion

Communication is essential for successful group-living. Competitive signals stabilize group structure through determining resource distribution or division of labor while cooperative signals maintain group integrity and coordinate activity. Tapping is a cooperative signal of *P. affinis*, and as such, may provide a mechanism for cohesion when used between a separated larva and a group. As predicted by (P1), groups respond to the tapping of separated larvae and do so preferentially over the other behaviors performed by the single individual. Additionally the groups truly respond as it is the single larva that initiates most of the behavioral activity and signal exchanges to which the group reciprocates.

Besides eliciting a response from the group, tapping (by the group) also increases the walking activity of the separated larva. Larvae spent significantly more time walking under experimental than control conditions. When on a tree, such an

increase in movement might serve to enhance a larva's chances of finding a conspecific or of reaching a branching point where it may obtain more specific directional information. Male tropical wandering spiders (*Cupiennius salei* Keyserling) use vibrational signals to locate females and often refine their movements at branching points by comparing signal strength between the two directions (Rover and Barth 1981). Perhaps this initial response of increased walking activity in the sawfly larvae enables them to further refine their search for the signaling group.

In addition to an overall increase in walking activity, larvae spent a higher proportion of time walking towards the center (in the direction of the group) when receiving signals in the experimental trials than during the 'quiet' control trials. Although the difference in proportion in the experimental trials was not quite significant ($p=0.053$), the result is most likely due to the small sample size. Insects from several different orders have the ability to obtain localization information from substrate vibrations, including other Hymenopterans (the order to which sawflies belong) (Virant-Doberlet et al. 2006), so it is reasonable to consider this function in pergid larvae.

Despite the widespread use of vibrational signals in insects, there are some challenges associated with localizing the source. Vibrations travel through the substrate in waves (bending waves being the most commonly used for communication) but they are often reflected, traveling up and down a plant several times before decaying below the detection threshold (Michelsen et al. 1982; Čokl and Doberlet 2003). This can make it difficult for the receiver to obtain directional information, especially for those insects that localize a signal based on detecting the arrival time differences of the component signal frequencies (Čokl and Doberlet 2003). Some insects, such as male leafhoppers (*Graminella nigrifrons*), solve the problem by employing more than one orientation cue. These males use a combination

of vibrational and visual cues when locating virgin females for mating (Hunt and Nault 1991).

Like the leafhopper, *P. affinis* larvae may employ a combination of cues for orientation and this requires further testing. Pergid larvae possess a pair of stemmata (simple eyes) that are capable of polarization sensitivity, motion detection, and visual resolution of high contrast objects as small as 4° (Meyer-Rochow 1974). In the current experimental set-up, the larva encountered two asymmetries. The first was the acoustic asymmetry being tested, where taps from the group came from the 'towards the center' direction of the branch. The second was a visual asymmetry where the 'towards the center' direction had a cardboard divider (15 cm2) around the branch while the 'edge' direction simply had the end of a branch. Thus, the separated larva could have used vibrational or visual cures, or both, to orient toward the center. It may be hypothesized that a separated larva did not detect vibrational signals from conspecifics and simply moved toward the origin of the stem for relocalizing its group. One could argue that the cardboard divider resembled the trunk of a tree and hence elicited movement towards it. The divider, however, was a square pattern and wandering arboreal larvae orient visually towards vertically extended patterns rather than square or circular patterns (Hundertmark, 1937). Moreover, separated control larvae did not move preferentially toward the cardboard divider. Thus, it is not likely that it played a role in orientation of the larva. Nevertheless, whether or not pergid larvae visually orient to vertically extended patterns representing a real tree trunk is a matter for future experimentation.

Regardless of whether larvae receive directional information solely from the vibrational signals or whether they use a combination of visual and vibratory cues for localization, it is clear that the vibrational signal elicits an increase in the general activity and search behavior of the separated larva. This alone is likely to facilitate

cohesion as it is the separated larva that increases its walking, not the grouped larvae. The grouped larvae also received tapping signals but instead of walking, their main response was tapping. While this study offers preliminary evidence regarding the receipt of directional information through tapping, it does not clearly distinguish between the use of vibratory signals and potential visual cues for orientation. Future experiments may address this directly by providing larvae with a uniform visual environment to which the vibrational stimulus is applied.

Why signal? Why cooperate? Given that tapping may facilitate cohesion, one may ask the obvious next questions…why does an individual signal and why does the group respond cooperatively? Single larvae of a gregarious species often fare quite poorly if removed from their group (e.g. Ghent 1960). The reasons vary according to the species involved but include factors such as reduced thermoregulatory capabilities (Seymour 1974; Klok and Chown 1999), exposure/dessication (Klok and Chown 1999), and reduced feeding (due to lack of group facilitation) (Ghent 1960; Nahrung et al. 2001; Reader and Hochuli 2003). No solitary *P. affinis* larvae have been observed in the field and experimental work with single larvae vs groups indicate a much higher mortality for single individuals (Fletcher, in review), both of which suggest a high fitness cost to being alone.

With clear fitness benefits to the individual when finding others, one next looks to understand how cooperation benefits the group. The evolution of cooperation has been explained in numerous ways including kin selection, by-product mutualism or reciprocity (Hamilton 1964; Axelrod and Hamilton 1981; Mesterton-Gibbons and Dugatkin 1992). Kin selection supports acts of altruism and cooperation by providing indirect genetic benefits to the cooperator; increasing the fitness of kin with shared genes acts to increase one's own fitness indirectly. In by-product mutualism, cooperation is a selfish phenomenon that benefits the cooperator directly while

secondarily providing benefits to the receiver; this often occurs in situations of harsh environments where neither party fares very well without cooperating. Reciprocation works in situations of repeated interactions of an undetermined number. By far the most robust strategy of reciprocation is 'Tit-for-Tat' (TFT) where an individual starts an interaction by cooperating and then copies its partner's previous strategy (cooperate or defect) in future interactions (Axelrod and Hamilton 1981).

A subsequent extension of the TFT strategy and theory for evolving cooperation is the Raise-the-Stakes (RTS) strategy. This basically acknowledges that cooperation is not an 'all-or-none' phenomenon and that one can incrementally increase its investment in cooperation based on the response of one's partner (Roberts and Sherratt 1998). For example, impala (*Aepyceros melampus*) engage in reciprocal allogrooming with partners but instead of performing one long grooming session on each other, they exchange shorter bouts and may lengthen their reciprocal bouts depending on the action of their partner (Hart and Hart 1992). Likewise, when looking at the cooperative investment of groups to a signaling larva in *P. affinis*, it appears as if they employ an RTS strategy. First of all, the number of taps a larva receives from the group is correlated with the number of taps given by that single larva (see Figure 2.5). This suggests that groups will not invest much in communicating with a separated individual unless the individual adequately and persistently signals its need. Secondly, the level of a group's investment in terms of the behaviors it performs (2 or more larvae tapping, group contraction, or walking) may be predicted based on the cumulative time the single larva has tapped in the trial (see Figure 2.6). Two or more larvae tapping is less of an investment than the entire group contracting (which indicates possible attempts to coordinate their activity, Fletcher 2007), and contracting is less of an investment than walking towards the signaler. Both of these results support the prediction (P4) that the group's response (i.e. investment) is

influenced by the single larva's behavior, and in doing so, they reflect usage of the RTS cooperative strategy.

Although groups may apply the RTS strategy for cooperation, this does more to address *how* rather than *why* they cooperate. Addressing ultimate requires looking at the fitness benefits of cooperating with or responding to an isolated larva. The fact that groups do respond to separated larvae suggests that they receive some benefit. One could invoke kin selection, at least in the early instars when colonies are composed of siblings, where increasing the fitness of a sibling increases one's own fitness indirectly. Because colonies readily coalesce with other unrelated groups and even other species (Carne 1962), this provides an inadequate explanation. The most convincing explanation may be cooperation evolving through interdependence (i.e. stakeholder's altruism) (Roberts 2005). Here, the altruist or cooperator has a stake in the recipient's welfare since its fitness is partially dependent upon or affected by that of the recipient. This is most commonly seen in cases where individual fitness is positively related to group size. For example, in cooperatively breeding meekrats, *Suricata suricatta*, small groups have lower fitness than large groups (Clutton-Brock et al. 2001b). Helpers, who feed the pups, have a stake in their welfare since pup survival increases group size (and this is true even when relatedness is low or zero; (Clutton-Brock et al. 2001a). If the benefits of being in a group for *P. affinis* increase with group size, then groups would have a stake in the signaler's welfare since that individual could provide another member for the colony.

Benefits that increase with numbers may include defense, foraging success, the dilution effect with parasitoids, and protection from desiccation. Larvae of *P. affinis* are chemically defended by storing eucalyptus oil in a diverticular sac, which is regurgitated upon disturbance. Morrow et al. (1976) demonstrated the oil's ability to repel potential arthropod and vertebrate predators and its effectiveness no doubt

increases with an increase in group size. Carne (1969) suggested a possible connection between group size and foraging success in his study on *P. affinis* population dynamics. He reported that few larvae survived on trees with low initial numbers while trees with higher densities of larvae had higher larval survivorship. Since colonies readily coalesce to form larger groups, this finding suggests that the ability to recruit other individuals and increase group size is important for overall survival. Although the exact reason for the enhanced survivorship is not known, Carne (1969) suggested that the coordination of movement and foraging activity was better in larger groups. Work on a related species, *Perga dorsalis*, showed that 20% of larvae in two colonies assumed 'leadership' positions more often than expected by chance (Weinstein and Maelzer 1997). Perhaps *P. affinis* is similar so that small groups may have fewer 'leaders' and therefore forage in a less coordinated or efficient manner.

At the later stages of development, Carne (1969) reported the major causes of mortality to be desiccation, parasitism, and fungal disease (of water-logged cocoons). Larvae persist in the winter and early spring so as the weather gets warmer and drier, desiccation becomes a serious threat, especially when larvae descend from the tree en masse to pupate. Carne (1969) reported a 40% mortality rate due to desiccation during soil entry and he also noted that colony size influenced soil entry success (large colonies were better able to penetrate the soil and did so at a faster rate). This again points to a benefit of colonies coalescing with others to form large aggregations prior to pupation. Higher numbers also help through the dilution effect with parasitoids as much parasitism occurs while the larvae descend to pupate (Carne 1969).

It is clear that, through increasing its overall size, a group may benefit in many ways by responding to a separated larva. The group's response represents cooperation through interdependence since by 'helping' the separated larva, they receive secondary

benefits. It is noteworthy that when larvae become sick or weak they often fall behind the group, becoming isolated (personal obs). Sick larvae rarely tap and if they do, they tap softly and slowly. It may be that the vigorous and relatively fast tapping of a separated larva not only signals its 'desire' to find the others but also provides an honest signal of the larva's health. Groups would have more to gain by recruiting a healthy, vigorous individual than a sick or weakened one.

Relative investments in exchanges. Although generally across organisms situations of cooperation positively affect both parties, in many cases there is an asymmetry in the benefits received such that one has more to gain than the other. Fur seals (*Callorhinus ursinus*) breed in large colonies and when a mother leaves to forage, she and her pup must reconnect upon her return. Both mother and pup use vocal cues for recognition but it is the pup that invests most of the energy in the reuniting process (Insley 2001). A successful reunion is good for each individual but it is crucial for the pup's survival (and hence its fitness) while representing only a portion of the female's lifetime reproductive fitness. Similarly, when a mother bottlenose dolphin (*Tursiops truncatus*) and her young are separated, they re-unite via acoustic vocalizations and it is the young that invests the most in this calling (Smolker et al. 1993).

With *P. affinis* we definitely have a cooperative exchange occurring between a signaling lone larva and a responding group. But again, (while both parties gain in the exchange), there is a clear asymmetry in the benefits received. A group of larvae already has a valuable resource and although it is augmented by increasing group size, such an addition is not vital for survival. However, re-connecting with a group is vital for a lone individual so one would expect the single larva to invest more in the exchange than the group, as predicted by P3. All three measures of investment (initiating exchanges, total taps per trial and rate of tapping) support the prediction indicating that the single larva, who has the most to gain, also invests the most in the

exchange.

Up until this point, I have discussed the two parties of the cooperative exchange as being a single larva and a group (as a complete entity). A group, however, is made up of individuals so although their behavior can be quantified aggregately, I can also look at the investment per individual. This was done to assess investment in signaling (taps per larva per trial) between the single larvae and grouped larvae but I can also compare the investment of individuals within a group. When examining the percentage of total taps given by each larva in 12 groups, it was clear that the tapping was not equally distributed among colony members (see Table 2.1), with at least two larvae doing the majority of the signaling.

In many cases the benefits of being in a group are not shared equally among colony members, and this often stems from a positional effect (Krause 1994). Membracid nymphs (*Umbonia crassicornis*) aggregate on their host plant and receive maternal care in the form of predator defense. Those nymphs closer to the mother receive better protection, and those on the perimeter have a higher risk of being attacked (Cocroft 2002). One could speculate that *P. affinis* larvae on the edge of the group are more likely to tap than those in the middle due to their increased exposure, thereby explaining the difference in tapping. This occurs during processions, where individuals at the back of the line (lacking posterior contact with other larvae) tap more than those leading the group (Fletcher 2007). The groups used in this study, however, were quite small (4-6 larvae), so that individuals were, in fact, equally exposed. Such a result may instead provide evidence of individual differences in behavior and propensities to tap, analogous to the leadership roles taken by certain individuals in the study by Weinstein and Maelzer (1997). One would have to see if the differences in tapping were stable over time but signaling behavior, as it is used to both coordinate activity and facilitate cohesion, could be seen as a leadership role.

In summary, like many group-living animals, *P. affinis* faces the challenge of maintaining a cohesive unit despite daily movements between foraging and resting sites. Tapping facilitates this cohesion when used between separated larvae and a group, as tapping elicits a response from the group, increases the walking activity of the separated individual, and may provide directional information to guide the individual towards the group. The exchange is a cooperative one with asymmetrical benefits leading to a difference in investment for the re-uniting process. Not only do groups invest less in the exchange but they appear to employ a RTS cooperative strategy by metering their behavioral response based on the cumulative signaling received from the individual. The example provided by *P. affinis*, and the range of other organisms that share similar strategies, point to the incredible breadth of biological complexity that employ "reciprocal communication of a cooperative nature" and hence, exhibit the essence of sociality as defined by Wilson (1971).

REFERENCES

Axelrod R, Hamilton WD (1981) The evolution of cooperation. Science 211:1390-1396

Boinski S (2000) Social manipulation within and between troops mediates primate group movement. In: Boinski S, Garber PA (eds) On the Move: How and Why Animals Travel in Groups. Chicago University Press, Chicago, pp 421-469

Boinski S, Mitchell CL (1992) Ecological and social factors affecting the vocal behavior of adult female squirrel-monkeys. Ethology 92:316-330

Carne P (1962) The characteristics and behaviour of the sawfly *Perga affinis affinis* (Hymenoptera). Australian Journal of Zoology 10:1-34

Carne P (1969) On the population dynamics of the eucalypt-defoliating sawfly *Perga affinis affinis* Kirby (Hymenoptera). Australian Journal of Zoology 17:113-141

Clutton-Brock TH, Brotherton PNM, O'Riain MJ, Griffin AS, Gaynor D, Kansky R, Sharpe L, McIlrath GM (2001a) Contributions to cooperative rearing in meerkats. Animal Behaviour 61:705-710

Clutton-Brock TH, Russell AF, Sharpe LL, Brotherton PNM, McIlrath GM, White S, Cameron EZ (2001b) Effects of helpers on juvenile development and survival in meerkats. Science 293:2446-2449

Cocroft RB (2002) Antipredator defense as a limited resource: unequal predation risk in broods of an insect with maternal care. Behavioral Ecology 13:125-133

Cokl A, Doberlet MV (2003) Communication with substrate-borne signals in small plant-dwelling insects. Annual Review of Entomology 48:29-50

Fitzgerald T, Peterson S (1988) Cooperative foraging and communication in caterpillars. BioScience 38:20-25

Fletcher LE (2007) Vibrational signals in a gregarious sawfly larva (*Perga affinis*): Group coordination or competitive signaling? Behavioral Ecology and Sociobiology 61:1809-1821

Fletcher LE (in review) The art of social-living: Examining selective benefits of grouping in a sawfly larva, *Perga affinis*.

Ghent AW (1960) A study of the group-feeing behavior of larvae of the jack pine sawfly, *Neodiprion pratti banksianae* Roh. Behaviour 16:110-147

Greenberg R (2000) Birds of many feathers: The formation and structure of mixed-species flocks of forest birds. In: Boinski S, Garber PA (eds) On the Move:

How and Why Animals Travel in Groups. University of Chicago Press, Chicago, pp 521-558

Hamilton WD (1964) The evolution of social behavior. Journal of Theoretical Biology 7:1-52

Hart BL, Hart LA (1992) Reciprocal allogrooming in impala, *Aepyceros melampus*. Animal Behaviour 44:1073-1083

Holekamp KE, Boydston EE, Smale L (2000) Group travel in social carnivores. In: Boinski S, Garber PA (eds) On the Move: How and Why Animals Travel in Groups. Chicago University Press, Chicago, pp 587-627

Hundertmark A (1937) Das Formenunterscheidungsvermögen der Eiraupen der Nonne (*Lymantria monacha* L.) Zeit Vergl Physiol 24:563–582

Hunt RE, Nault LR (1991) Roles of interplant movement, acoustic communication, and phototaxis in mate-location behavior of the leafhopper *Graminella nigrifrons*. Behavioral Ecology and Sociobiology 28:315-320

Insley SJ (2001) Mother-offspring vocal recognition in northern fur seals is mutual but asymmetrical. Animal Behaviour 61:129-137

Jolivet P, Vasconcellos-Neto J, Weinstein P (1990) Cycloalexy: a new concept in the larval defence of insects. Insecta Mundi 4:133-142

Jones IL, Falls JB, Gaston AJ (1987) Vocal recognition between parents and young of ancient murrelets, *Synthliboramphus antiquus* (Aves, Alcidae). Animal Behaviour 35:1405-1415

Kalbfleisch JD, Prentice RL (2002) The Statistical Analysis of Failure Time Data, 2 edn. John Wiley & Sons, Inc., Hoboken

Klok CJ, Chown SL (1999) Assessing the benefits of aggregation: thermal biology and water relations of anomalous Emperor Moth caterpillars. Functional Ecology 13:417-427

Krause J (1994) Differential fitness returns in relation to spatial position in groups. Biological Reviews of the Cambridge Philosophical Society 69:187-206

Markl H (1965) Stridulation in leaf-cutting ants. Science 149:1392-1393

Mesterton-Gibbons M, Dugatkin LA (1992) Cooperation among unrelated individuals - Evolutionary factors. Quarterly Review of Biology 67:267-281

Meyer-Rochow VB (1974) Structure and function of larval eye of sawfly, *Perga*. Journal of Insect Physiology 20:1565-1591

Michelsen A, Flemming F, Gogala M, Traue D (1982) Plants as transmission channels for insect vibrational songs. Behavioral Ecology and Sociobiology 11:269-281

Morrow P, Bellas T, Eisner T (1976) Eucalyptus oils in the defensive oral discharge of Australian sawfly larvae (Hymenoptera: Pergidae). Oecologia 24:193-206

Nahrung HF, Dunstan PK, Allen GR (2001) Larval gregariousness and neonate establishment of the eucalypt-feeding beetle *Chrysophtharta agricola* (Coleoptera: Chrysomelidae: Paropsini). Oikos 94:358-364

Ota D, Cokl A (1991) Mate location in the southern green stink bug, *Nezara viridula* (Heteroptera, Pentatomidae), mediated through substrate-borne signals on ivy. Journal of Insect Behavior 4:441-447

Raper KB (1984) The Dictyostelids. Princeton University Press, Princeton

Reader T, Hochuli DF (2003) Understanding gregariousness in a larval Lepidopteran: the roles of host plant, predation, and microclimate. Ecological Entomology 28:729-737

Roberts G (2005) Cooperation through interdependence. Animal Behaviour 70:901-908

Roberts G, Sherratt TN (1998) Development of cooperative relationships through increasing investment. Nature London 394:175-179

Rovner JS, Barth FG (1981) Vibratory communication through living plants by a tropical wandering spider. Science 214:464-466

Seymour R (1974) Convective and evaporative cooling in sawfly larvae. Journal of Insect Physiology 20:2447-2457

Smolker RA, Mann J, Smuts BB (1993) Use of signature whistles during separations and reunions by wild bottle-nosed-dolphin mothers and infants. Behavioral Ecology and Sociobiology 33:393-402

Trivers R (1985) Social Evolution. The Benjamin/Cummings Publishing Company, Inc., Menlo Park

Virant-Doberlet M, Cokl A, Zorovic M (2006) Use of substrate vibrations for orientation: From behavior to physiology. In: Drosopoulos S, Claridge MF (eds) Insect Sounds and Communication. Taylor and Francis, Boca Raton, pp 81-97

Weinstein P, Maelzer D (1997) Leadership behaviour in sawfly larvae *Perga dorsalis* (Hymenoptera: Pergidae). Oikos 79:450-455

Wilson EO (1971) The Insect Societies. Belknap Press of Harvard University Press, Cambridge

CHAPTER 3

SAME SIGNAL, DIFFERENT MEANING? TESTING ALTERNATE FUNCTIONS OF TAPPING IN THE AUSTRALIAN SAWFLY LARVA, *PERGA AFFINIS*

Abstract

In group-living organisms, communication serves a range of functions. While some species have large repertoires to accommodate several functions, others are more limited, requiring alternate ways of assigning signal meaning. Two such methods involve using a signal's context or changing a signal's parameters, such as rate, to differentiate between meanings. Larvae of the Australian sawfly, *Perga affinis*, live in groups and communicate via two vibrational signals, contractions and tapping. For tapping, context plays a role in defining at least two functions. During processions, tapping by back-positioned larvae directs movement forward while, in the context of separation, it serves as a call-and-response signal for an isolated larva. This study examines whether or not tapping serves a third function as a 'come here' or recruitment signal where the meaning is differentiated based on signal rate. I test this in a playback experiment with three treatments: a high tapping rate (PB1), low tapping rate (PB3), and a control. Larvae responded to the playbacks through increased tapping; however, contrary to expectation, the high tapping rate had an inhibitory effect on turning towards the stimulus. Instead of interpreting the playback as a recruitment signal from a separated larva, they may have viewed it as a back-positioned coordination signal and hence, continued to move forward. I discuss the results in terms of the sensory perceptions of signal receivers and the multidimensional role that context plays in providing information during communication exchanges.

Introduction

Among group-living organisms, communication plays an essential role in coordinating activities and maintaining a cohesive unit. Certain signals enable members of a group to indicate readiness to depart, such as piping in honey bee swarms, grunts in resting gorillas, and wing-flapping along with vocalizations in water-borne swans (Visscher & Seeley 2007; Stewart & Harcourt 1994; Black 1988, respectively). Other signals such as contact calls, provide a way for group members to stay connected during travel or movement. This is especially true in conditions of impaired vision such as foraging in a forest, across large distances or underwater (Poole et al 1988; Janik & Staler 1998; Radford 2004a). In instances where individuals are separated from the group, such as after solitary foraging, animals often signal as a way to find the group and re-aggregate (Braune et al 2005; Seddon et al 2002).

Social organisms achieve this range of communicative functions in several ways. Some have large repertoires, enabling them to devote signal types to specific functions. Others have more limited repertoire sizes, but signal function may be determined by who receives it (receiver context) or by the environmental context. Male fiddler crabs (*Uca perplexa*) have a vertical wave display where function depends on receiver context. Between males, waving is a territorial signal. However, when females are in close range, waving is a courtship signal (How et al. 2007). Saddle-backed (*Saguinus fuscicollis*) and emperor tamarins (*S. imperator*) employ a single call type, long calls, for two different functions that depend upon the environmental context. While traveling, it acts as intra-group contact call but during encounters with neighboring clans, it serves as an inter-group territorial signal (Windfelder 2001).

A second method of assigning meaning involves changing signal parameters, such as rate, to convey a difference in information. Rate can vary along a continuum,

allowing it to encode gradations of information such as proximity to danger in alarm calls or level of food deprivation in nestling begging calls (Beletsky 1991; Leonard & Horn 2001; Warkentin et al. 2001). In other instances, a change of rate may actually alter the signal's function, as seen in the 'kek' vocalization of green woodhoopoes (*Phoeniculus purpureus*); a low calling rate functions as a contact call when birds forage, while a higher call rate is used to repel competitors (Radford 2004b).

Group-living larval insects have physiological constraints on their capacity for signal production. Larvae have a conserved body type and lack the common acoustic signal-producing structures seen in adult insects such as scrapes and files or stridulating legs and wings. Many rely on chemical communication, but even here, signal production is generally limited to a trail pheromone (Weyh & Maschwitz 1978; Capinera 1980; Fitzgerald & Peterson 1988; Roessingh 1990), although a few species also have a recruitment signal (Fitzgerald 1976; Peterson 1988). Visual signals may be important in predator deterrence but are unlikely to be used for intra-group communication as larvae have only simple stemmata with relatively low resolving power (Gullan & Cranston 2000). Despite these constraints, group-living larvae are under strong selective pressures to maintain a cohesive unit and hence, communicate. The need for communication coupled with their simpler body plan, make social larvae an interesting group for investigating the function and use of signals in the context of a limited repertoire.

This study examines vibrational signal use in a gregarious Australian sawfly larva, *Perga affinis*. Larvae of *P. affinis* live in sibling colonies, forming tight, inactive clusters during the day while dispersing to feed at night. As a group-living, processionary species, they require communication for coordinating activity, providing directional information, finding others if separated, advertising food, and re-aggregating after feeding. To accomplish these tasks, *P. affinis* larvae rely on two

vibrational signals, contractions and tapping. Contractions consist of a full-bodied twitch that is both vibatrional and tactile in nature; tapping involves striking the sclerotized tip of the abdomen on the substrate.

Tapping occurs in a variety of contexts, suggesting that its function may vary accordingly. The first context is that of separation, where tapping acts as a call-and-response signal or a separation call. Larvae are much more likely to tap when alone than when in a group and the tapping of a single larva elicits tapping in others (Fletcher 2007). Additionally, when engaged in a tapping exchange, a single larva initiates the exchange and invests more in signaling than the group (Fletcher 2008), both of which are expected as the individual gains more by joining the group than the group does by receiving it. The second context for tapping involves processions, which consist of a cyclic pattern of movement punctuated by periods of stillness. During the periods of movement, a wave of contractions moves from the front to the back of the colony, followed by tapping, and then walking. As tapping occurs prior to walking and as back-positioned larvae tap significantly more than those in the front (Fletcher 2007), in the procession context, tapping likely functions to coordinate movement and to direct larvae forward.

In addition to serving as a separation call and coordination signal, tapping may have a third function of leading larvae or providing directional information to the group. The context for this function is more variable, suggesting that a continuous parameter such as rate may be the key factor in identifying the signal's function. The idea for this function stems from observations in two situations. First, occasionally I have witnessed lone larvae tapping while feeding on a leaf until joined by at least 2-3 other individuals, suggesting a 'come here' function. Secondly, during processions I have witnessed a few individuals proceeding up a side branch and tapping as if calling the group to follow them. In both cases, the signalers have had variable recruitment

success. It may be that the rate of tapping bouts indicates a signaler's persistence where a higher rate of tapping changes the signal from a call-and-response/separation call to a 'come here' signal.

This study examines tapping and its functions through the use of playback experiments. The aims are 2-fold. The first is to determine whether or not the larvae respond to playbacks of tapping by comparing the signaling behavior of processing larvae between control and playback trials. As tapping of a lone larva has been shown to elicit tapping in others (Fletcher 2007), I predict that larvae will tap more during the playback trials than during the controls. Additionally, as there will be two playback treatments: a high tapping rate (1 bout of tapping per minute, PB1) and a lower rate (1 bout of tapping every 3 minutes, PB3) and since a group's tapping response is influenced by the tapping investment of the lone signaler, I predict that larvae will tap more in PB1 than PB3. If larvae do respond to playbacks, the second aim involves examining how the rate of the tapping signal affects the groups' behavior and thus, the signal's function. If a higher rate of tapping changes the signal from a call-and-response to a 'come here' signal, I predict that more larvae will change direction during the procession towards the playback stimulus in PB1 than PB3 and more in the playback than the control treatments.

Methods

Three colonies of about 20 larvae each were collected from the field in the Australian Capital Territory, 20 km outside of Canberra. I split the larvae into nine groups of five individuals and maintained them on fresh eucalyptus cuttings in the laboratory. Larvae resided in their assigned groups undisturbed for at least 3 days prior to experimental testing, thereby allowing them to reestablish their normal feeding and activity patterns. At the time of testing, most larvae were in their 4^{th} instar.

Experimental Design. All trials took place on a straight 55cm branch of eucalyptus from which I removed all leaves and smaller side branches. Use of a single branch allowed me to minimize variability in the playback signals between trials since substrate properties such as wood hardness, branch diameter, etc. affect the signal's propagation (Markl 1983). Because much of *P. affinis'* vibrational signaling occurs during periods of group movement, I conducted all trials on groups that were actively processing (i.e. walking from a resting site to a feeding location, see example in Figure 3.1). Once a group began processing on its original cutting, I transferred it to the test branch, taking care to place the larvae in the same order and position. Trials did not begin until the group resumed processing and was a distance of 10 cm from the point of vibrational stimulation.

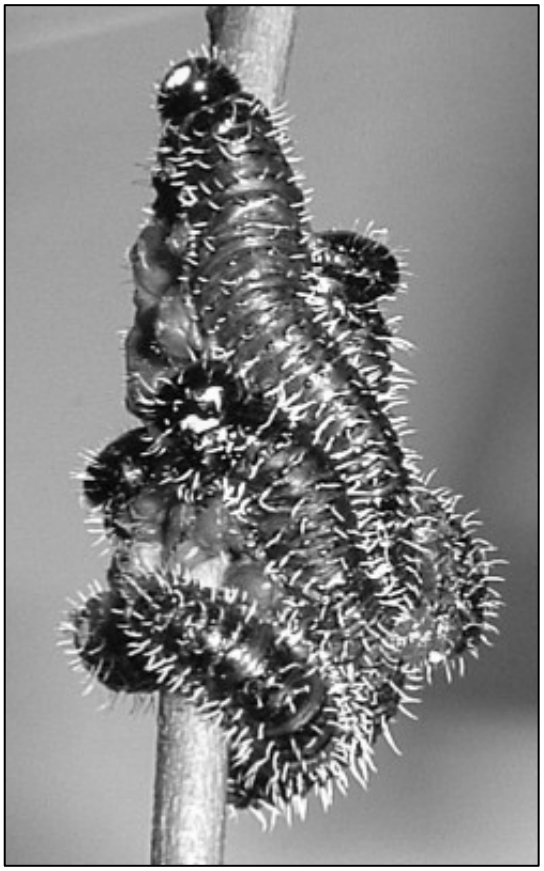


Figure 3.1 A small experimental colony of larvae processing along a branch. Scale bar: 5mm.

Each of the nine groups underwent three treatment conditions (order of presentation was randomized): a control and two playback conditions. Trials lasted for 30 minutes. The first playback condition (PB1) involved presenting an 8-second tapping bout of 24 taps at 1-minute intervals (giving 52 seconds of silence between each bout). The second playback condition (PB3) involved the same 8-second tapping stimulus but it was played back at 3-minute intervals (giving 172 seconds of silence between each bout). Thus the two playback conditions differed in their rates of tapping bouts, which simulated differing levels of persistence in tapping by an outside signaler as well as different investment levels (in terms of total number of taps) by the signaler.

Both of the intervals between tapping bouts (i.e. tapping bout rates) were within the natural range for *P. affinis* larvae. This was determined by recording the tapping activity of 20 solitary larvae on a single branch over the course of 20 minutes. I then quantified the length of time between each bout of tapping and calculated a median interval length for each individual. For the 20 larvae the interval length ranged from 1-598s, with the median interval for an individual ranging from 13-110.5s and the overall median interval being 54.77s. Therefore, PB1 is the faster rate of the two playbacks and more closely resembles the median tapping bout rate of the larvae.

A total of 3 recorded tapping signals (each 8 seconds in length with 24 taps) were used as playback stimuli. I randomly assigned one of the three taps to each group so that a group received the same tapping signal in both of its playback conditions. During control trials, no signal was played; however, the needle attached to the playback speaker remained in contact with the branch, thereby allowing any electrical background noise to be transmitted to the substrate. This controlled for any potential effect of the background noise on the groups' behavior.

Acoustic recording and playback set-up. Tapping signals of three different larvae were recorded for use in the playback trials. Recordings were made at a known distance from a Knowles accelerometer (model BU 1771) that was connected to a preamplifier and then to a PC laptop computer. Using Raven 1.1 software (Cornell Laboratory of Ornithology), I digitally captured the tapping signals onto the computer where the peak amplitude and RMS values for the signals were recorded for assessing the correct playback volume of the tapping stimuli. Playbacks occurred by playing the recorded signal through the computer to a preamplifier that was attached to an Audax cone speaker (#AP080M4) with a needle at its center. The needle vibrated in response to the sound and by placing it in contact with the branch, it transmitted the vibrational signal to the substrate. All signals were played at a distance of 10 cm from the marked starting point of the trial (a set location to which the larvae processed before the trial began). Playback signals were calibrated by recording the needle's output at a distance of 10 cm from the accelerometer. By adjusting the phono and volume setting on the preamplifier, I could reproduce the playback signals at biologically relevant levels that were equal in amplitude for all trials.

All trials (3 treatments/group) were videorecorded with a Sony Digital 8 DCR-TRV830 camcorder for detailed behavioral analysis. By attaching an accelerometer to the experimental branch and connecting it to the preamplifier and videocamera, I also simultaneously recorded the vibrational signals of the larvae. I collected data on the group as a whole as well as on individuals since each larva was separately marked with colored model paint on the back of its head capsule and on the sclerotized tip of its abdomen.

Examining Aim 1: Do larvae respond to playbacks of the tapping signal? To address the first aim of the study, I compared the larvae's tapping behavior between control and playback situations. Tapping by a single larva has been shown to elicit

tapping in others (Fletcher 2007) so I predicted that larvae would tap more during playback than control trials. Additionally, since the magnitude of a group's response (i.e. total number of taps) is correlated to the number of taps given by the signaler (Fletcher 2008), I also predicted that larvae would tap more in PB1 - where they receive more tapping signals - than PB3. These first two predictions deal with tapping in its separation/call-and-response function but since the larvae were processing during the trials, I also tested for the coordination/"conductors from the back" function of tapping. I predicted that larvae would retain the positional effect of tapping, where back-positioned larvae tapped more than those in front.

To test these predictions, I first analyzed the data at the individual level. I divided the 30-minute trial into 7 time periods and recorded the total number of taps for each larva in every time period. I also recorded the individual's position within the group (front, middle or back) at each time period. Due to the left-skewed distribution of these count data, the data were analyzed in two steps. First, I conducted a multilevel binary logistic regression (xtmelogit in Stata v.10) for the presence or absence of tapping. The independent variables were treatment, position in the group, time in the trial, and number of individuals in the group (note – all groups started with 5 individuals but some died over the course of the experiment giving a range of 3-5 larvae in a group). The two random variables were group and individual. The second stage of analysis involved removing all cases where larvae did not tap and then conducting a multilevel Poisson regression (xtmepoisson in Stata v.10) on the number of taps. These count data were Poisson distributed so the analyses allowed me to determine if any differences occurred between treatments when individuals did tap. Here, the independent and random factors are the same as those stated for the binary regression above.

Examining Aim 2: Does the rate of tapping bouts affect the signal's function?

Here, I was particularly interested in whether or not the rate of tapping bouts (i.e. a signaler's persistence) changed tapping from a call-and-response to a 'come here' signal that encouraged others from the group to move towards the signaler. This differs from the separation/call-and-response function where it is the single larva that ends up finding its way towards the group. I tested Aim 2 by comparing the odds of a larva within the group changing direction (moving towards the playback stimulus) in the three treatments using a binary logistic regression (Stata v. 10). The independent variables were treatment (PB1, PB3, C) and group size. The two random variables were group and individual. I predicted that the odds of a larva turning around would be higher during playback than control trials and higher in PB1 than PB3.

Results

Aim 1: Do larvae respond to playbacks of tapping? Larvae demonstrated a clear tapping response to the playback stimuli. The odds of an individual tapping were significantly greater during the playback treatments than during the control (Figure 3.2). Similarly, when an individual did tap, there was a significant difference in the number of taps between the control and playback treatments, with the expected number of taps increasing by a factor of 1.91 and 1.89 for PB1 and PB3, respectively, compared to the control (Figure 3.2). There was no difference, however, in the expected number of taps between the two playback treatments, PB1 and PB3. Therefore, while playbacks elicited additional tapping by the larvae, the total number of taps produced by the playback in a trial - PB1: 240 vs PB3: 80 - did not affect the magnitude of the larvae's response.

Besides treatment, the other predictor variables in the Poisson regression included time during the trial, an individual's position in the group, and group size.

Time during the trial had no effect on tapping but an individual's position within the group significantly affected the expected number of taps (Figure 3.3). Those in back tapped significantly more than those in the middle or front (Poisson regression $Z=13.64$ $p<0.001$ and $Z=11.21$ $p<0.001$, respectively), indicating that larvae retained the coordination/"conductors from the back" function of tapping while processing during the trials. Size of the group also had a significant effect on the model, with the expected number of taps increasing by a factor of 1.62 for every additional group member (see Figure 3.4; Poisson regression $Z=19.58$ $p<0.001$).

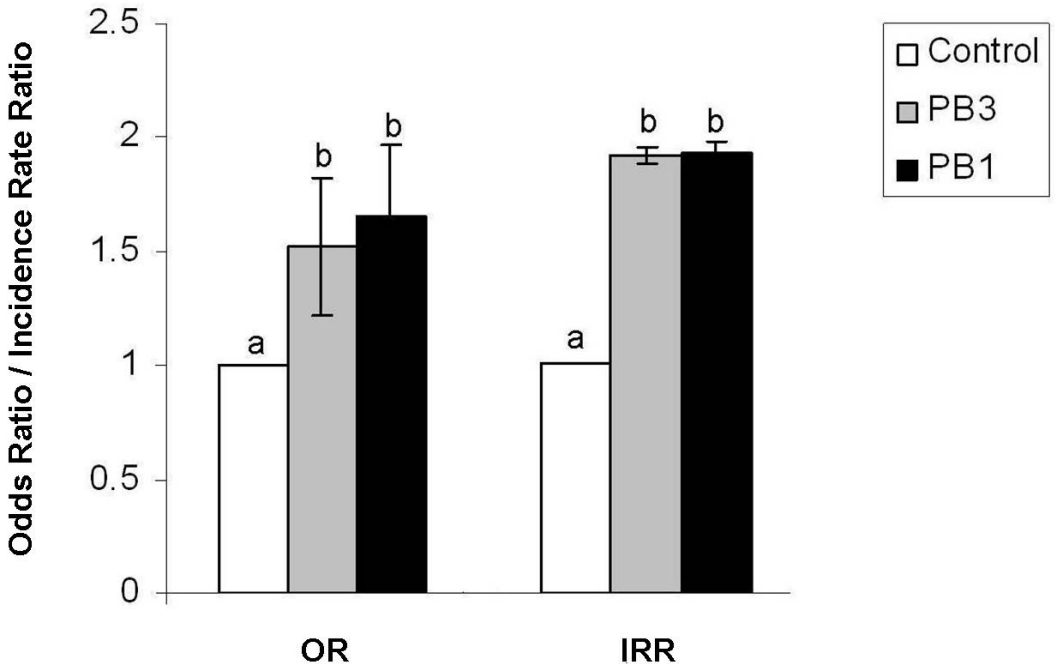


Figure 3.2 Odds ratio (OR) and incident rate ratio (IRR) for tapping in the three treatments (control – white, PB3 – grey, PB1 – black). The OR refers to the odds of a larva tapping in the two playback treatments relative to the control while the IRR compares the expected number of taps from a larva between the playback and control treatments; both measures were significantly higher in the playback than control treatments (Binary logistic regression, $Z=2.14$, $p=0.032$ for PB3 and $Z=2.65$, $p=0.008$ for PB1; Poisson regression, $Z= 29.63$ for PB3, $Z=31.87$ for PB1, $p<0.0001$).

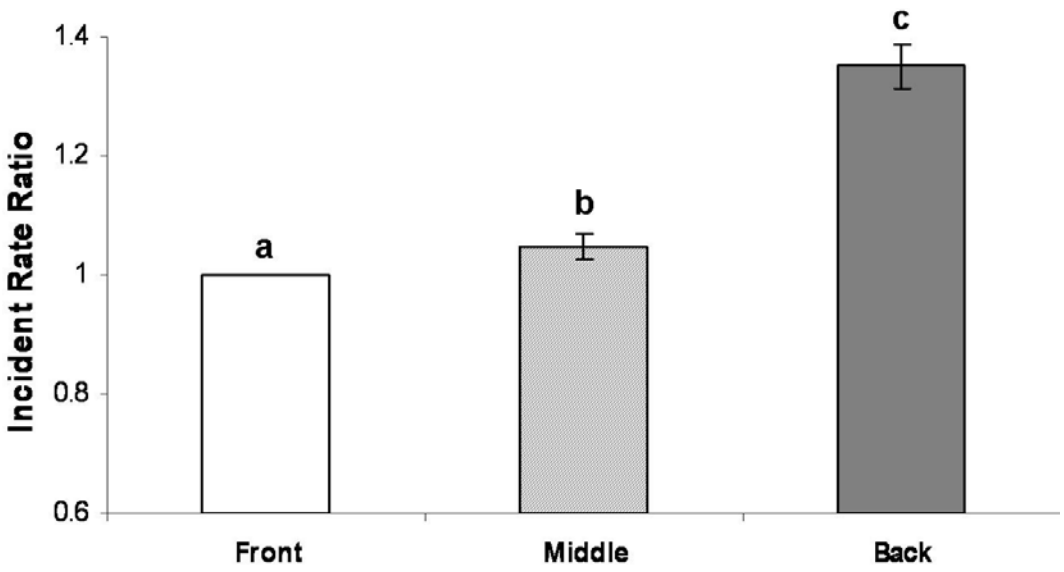


Figure 3.3 Incident rate ratio (IRR) for the expected number of taps by a larva based on its position within the processing group. Back-positioned larvae had a significantly higher number of expected taps than middle or front positioned individuals. All IRR values are relative to the front position (Poisson regression, $b = 0.031$, $c < 0.0001$).

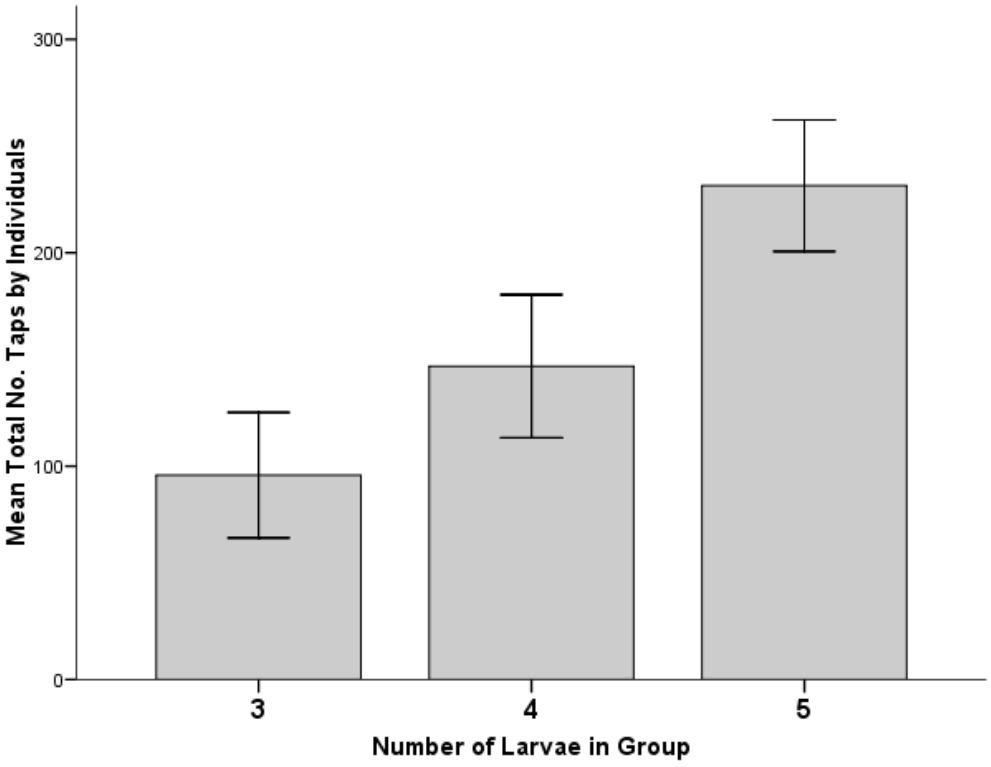


Figure 3.4 Mean total number of taps by individual larvae within different-sized groups. Error bars represent 1 STD. Group size was a significant factor in the model (Poisson regression, $Z = 19.78$ $p < 0.0001$).

Aim 2: Does the rate of tapping bouts affect the signal's function? Rate does affect the signal's function but not in the way predicted. My prediction stated that a high tapping bout rate would change the signal's function to a 'come here' signal, causing more larvae to turn towards the signal stimulus in PB1 than PB3 or the control trials. The results showed no significant difference in the odds of turning between the control and either PB1 or PB3 (Binary logistic regression, $Z=-1.49$, $p=0.136$ and $Z=1.63$, $p=0.103$, respectively). However, a comparison between the two playback treatments indicated that the odds of turning towards the stimulus were significantly lower during PB1 than during PB3 (see Figure 3.5; Binary logistic regression $Z=2.72$, $p=0.007$). Therefore, instead of PB1's high bout rate serving a 'come here' function, it appeared to have the opposite effect by reducing the odds of turning in larvae relative to the PB3 treatment. Although neither PB1 nor PB3 were significantly different from the control, they were significantly different from each other, suggesting that rate had some effect on whether or not the larvae turned towards the stimulus. Group size also had a significant negative effect on changing direction towards the stimulus, with the odds of turning decreasing by a factor of 0.332 for each additional group member (Binary logistic regression $Z=-2.29$, $p=0.022$).

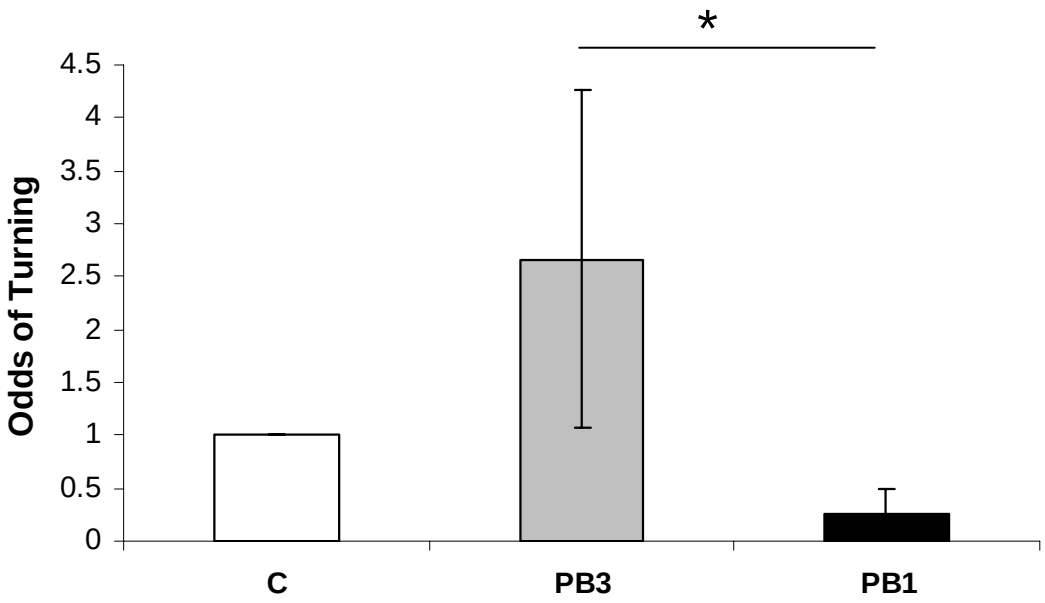


Figure 3.5 Odds of turning towards the tapping stimulus. Neither PB1 nor PB3 were significantly different from the control; however, the odds of turning were significantly lower during the PB1 than PB3 treatment (Binary logistic regression, $Z=-2.72$, $p = 0.007$).

Discussion

Aim 1: Do larvae respond to playbacks of tapping? As predicted, the tapping stimulus elicited a tapping response by the group members. Larvae had both higher odds of tapping and a higher number of expected taps during the playback trials than the control. Contrary to prediction, however, the expected number of taps given by larvae did not differ between the two playback treatments, PB1 and PB3, suggesting that they did not base their tapping response on the signaler's investment level.

Also contrary to expectation is the finding that group size influenced the number of taps produced by individuals within these groups. Larvae increased their tapping by a factor of 1.62 for each additional group member so that individuals in larger groups tapped more than those in smaller groups (see Fig 3.4). Group size had been added to the model simply to control for the fact that some colonies had fewer larvae than others due to sickness or death during the experiment. This result suggests that tapping may have a synergistic effect on other larvae. In a separation context, tapping by a lone larva elicits tapping in others; therefore, in a group context, it appears that the tapping of a larva's immediate neighbors also elicits more signaling. Such synergy may serve to reinforce the signaling of group members as seen in other chorusing species (Cocroft 2002, 2005). Synergy could be considered cooperative if signaling provides benefits for the entire group (Cocroft 2001). *Perga affinis* larvae do benefit from recruiting others to their group or maintaining group cohesion through signaling as survivorship of single larvae is much lower than survivorship of those in groups (Carne 1969; Fletcher unpublished data) due to increased foraging efficiency (Ghent 1960; Lawrence 1990), thermoregulation (Seymour 1974; Klok 1999; Ruf & Feilder 2000) and enhanced defense (Prop 1960; Tostowaryk 1972; Vulinec 1990; Boeve 1991) when grouped.

Aim 2: Does the rate of tapping bouts affect the signal's function? Many species

use signal rate to define alternate functions and, in certain communicative contexts, a high rate of signal production specifically serves a 'come here' or recruitment function as seen in spider monkeys (Chapman & Lefebvre 1990), white-faced capuchins (Boinski & Campbell 1995), and Eastern tent caterpillars (Fitzgerald 1993). Thus, I hypothesized that signal rate may affect the function of sawfly larva tapping in a similar manner. However, the results do not support the hypothesis. There was no significant difference in the odds of turning towards the stimulus between either of the playback conditions and the control. When comparing between the two playback treatments, the odds of turning were significantly lower in the high tapping rate treatment (PB1) than the low tapping rate treatment (PB3). This is the exact opposite of what had been predicted and suggests that the high tapping bout rate, instead of soliciting turning behavior, may have had an inhibiting effect.

As the playback stimulus was placed on the bottom of the larval group branch, only 10 cm behind the starting location of the group, it is possible that this simulated the tapping of a rear-positioned larva rather than a separated larva. Normally, tapping by back-positioned larvae has a coordinating effect on processions by encouraging the colony to move forward. The relentless tapping from the rear during PB1 (every minute) might have been interpreted in this way, thus inhibiting any turning behavior. Additionally, while the interval of PB1 was set up to reflect the common interval between tapping bouts of a separated larva, the silent period also corresponds to the average period of stillness in between activity bouts during a procession (ave = 53.7s; Fletcher 2007).

If larvae are in fact interpreting the playbacks as a rear-positioned larva rather than a separated, potentially recruiting individual, we would expect to see an increase in the amount of tapping from larvae during the playback as compared to control trials due to the perception of an additional group member. This may explain why there was

a higher number of taps during the playback treatments than the control but no difference between the high tapping rate, PB1, and the low rate, PB3. Due to a perceived increase in group size, the playback's representation of an 'additional larva' may have had a synergistic effect on the tapping of other group members and its effect may have occurred regardless of the rate or overall numbers of taps given in the playback.

These results highlight the importance of context in conveying a signal's message while also suggesting that organisms may use several lines of information to guide their behavior. For *P. affinis* larvae, the context of signaling, the direction from which the signal comes as well as interval duration may all contribute information to signal meaning. Although I originally intended to explore solely the effect of tapping rate on signal function, the results clearly suggest that larvae integrate different types of information. Much recent work on communication has focused on the use of multimodal signals as it is known that "animals communicate with their entire bodies and perceive signals with all available faculties" (Partan & Marler 2005, p. 231). While tapping itself is not a multimodal signal, it seems that receivers of the signal use information from both the signal properties and from the context in which the signal is given to assign the correct meaning. Additionally, context itself may be a multidimensional source of information as inferred by Leger's review (1993) on context in animal communication. He divides context into two broad categories: those dealing with the recipient and those dealing with the external environment. During the playback experiment, it appears that the recipient's context of processing coupled with the external context of signal's location influenced the tapping to be perceived as a within-group coordination signal rather than a signal from a separated larva.

If context itself provides a multidimensional source of information, it has the potential to greatly reduce the need of large repertoires sizes. In some social

vertebrates, repertoire size is positively correlated with social complexity (McComb & Semple 2005; Freeberg 2006; May-Collado et al 2007) but this is not true for all organisms (Blumstein & Armitage 1997) and suggests that there are many ways to communicate effectively without relying on repertoire size, per se. Additionally, we have seen that altering signal parameters such as rate provides ways to diversify a repertoire (Beletsky 1991; Leonard & Horn 2001; Warkentin et al. 2001). Although the experimental results with *P. affinis* do not provide clear evidence of rate conveying different signal functions, they also do not exclude this possibility. It may be necessary to repeat this test with a more refined and realistic set-up of a separation during a procession; this would require the addition of side branches with potential food sources as well as the placement of the playback stimulus on a side branch. Such a set-up accounts for other sources of information that are available to the larvae when they assess the meaning of the playback signal.

In closing, the interpretation of these experimental results reminds us of how even those organisms that appear simple, have lives rich in detail. The subtleties and nuances of communication may exist even at the finest of scales.

REFERENCES

Beletsky LD (1991) Alert calls of male red-winged blackbirds - Call rate and function. Canadian Journal of Zoology-Revue Canadienne De Zoologie 69:2116-2120

Black JM (1988) Preflight signaling in swans - A mechanism for group cohesion and flock formation. Ethology 79:143-157

Blumstein DT, Armitage KB (1997) Does sociality drive the evolution of communicative complexity? A comparative test with ground-dwelling sciurid alarm calls. American Naturalist 150:179-200

Boeve JL (1991) Gregariousness, field distribution and defense in the sawfly larvae *Croesus varus* and *C. septentrionalis* (Hymenoptera, Tenthredinidae). Oecologia 85:440-446

Boinski S, Campbell AF (1995) Use of trill vocalizations to coordinate troop movement among white-faced capuchins - A 2nd field test. Behaviour 132:875-901

Braune P, Schmidt S, Zimmermann E (2005) Spacing and group coordination in a nocturnal primate, the golden brown mouse lemur (*Microcebus ravelobensis*): the role of olfactory and acoustic signals. Behavioral Ecology and Sociobiology 58:587-596

Capinera JL (1980) A trail pheromone from silk produced by larvae of the range caterpillar *Hemileuca oliviae* (Lepidoptera, Saturniidae) and observations on aggregation behavior. Journal of Chemical Ecology 6:655-664

Carne P (1969) On the population dynamics of the eucalypt-defoliating sawfly *Perga affinis affinis* Kirby (Hymenoptera). Australian Journal of Zoology 17:113-141

Chapman CA, Lefebvre L (1990) Manipulating foraging group-size - Spider monkey food calls at fruiting trees. Animal Behaviour 39:891-896

Cocroft RB (2001) Vibrational communication and the ecology of group-living, herbivorous insects. American Zoologist 41:1215-1221

Cocroft RB (2002) Antipredator defense as a limited resource: unequal predation risk in broods of an insect with maternal care. Behavioral Ecology 13:125-133

Cocroft RB (2005) Vibrational communication facilitates cooperative foraging in a phloem-feeding insect. Proceedings of the Royal Society of London B-Biological Sciences 272:1023-1029

Fitzgerald T, Peterson S (1988) Cooperative foraging and communication in caterpillars. BioScience 38:20-25

Fitzgerald TD (1976) Trail marking by larvae of eastern tent caterpillar. Science 194:961-963

Fitzgerald TD (1993) Trail following and recruitment - Response of Eastern tent caterpillar *Malacosoma americanum* to 5-beta-cholestane-3,24-dione and 5-beta-cholestan-3-one. Journal of Chemical Ecology 19:449-457

Fletcher LE (2007) Vibrational signals in a gregarious sawfly larva (*Perga affinis*): Group coordination or competitive signaling? Behavioral Ecology and Sociobiology 61:1809-1821

Fletcher LE (2008) Cooperative signaling as a potential mechanism for cohesion in a gregarious sawfly larva, Perga affinis. Behavioral Ecology and Sociobiology 62:1127-1138

Freeberg TM (2006) Social complexity can drive vocal complexity: Group size influences vocal information in Carolina chickadees. Psychological Science 17:557-561

Ghent AW (1960) A study of the group-feeing behavior of larvae of the jack pine sawfly, *Neodiprion pratti banksianae* Roh. Behaviour 16:110-147

Gullan PJ, Cranston PS (2000) The insects: An outline of entomology, 2 edn. Blackwell Science, Oxford; Malden, MA

How MJ, Zeil J, Hemmi JM (2007) Differences in context and function of two distinct waving displays in the fiddler crab, *Uca perplexa* (Decapoda : Ocypodidae). Behavioral Ecology and Sociobiology 62:137-148

Janik VM, Slater PJ (1998) Context-specific use suggests that bottlenose dolphin signature whistles are cohesion calls. Animal Behaviour 56:829-838

Klok CJ, Chown SL (1999) Assessing the benefits of aggregation: thermal biology and water relations of anomalous Emperor Moth caterpillars. Functional Ecology 13:417-427

Lawrence WS (1990) The effects of group size and host species on development and survivorship of a gregarious caterpillar *Halisidota caryae* (Lepidoptera: Arctiidae). Ecological Entomology 15:53-62

Leger DW (1993) Contextual sources of information and responses to animal communication signals. Psychological Bulletin 113:295-304

Leonard ML, Horn AG (2001) Acoustic signalling of hunger and thermal state by nestling tree swallows. Animal Behaviour 61:87-93

Markl H (1983) Vibrational communication. In: Huber F, Markl H (eds)

Neuroethology and Behavioral Physiology: Roots and growing points. Springe-Verlag, New York, pp 334-353

May-Collado LJ, Agnarsson I, Wartzok D (2007) Phylogenetic review of tonal sound production in whales in relation to sociality. BMC Evolutionary Biology 7

McComb K, Semple S (2005) Coevolution of vocal communication and sociality in primates. Biology Letters 1:381-385

Partan SR, Marler P (2005) Issues in the classification of multimodal communication signals. American Naturalist 166:231-245

Peterson SC (1988) Chemical trail marking and following by caterpillars of *Malacosoma neustria*. Journal of Chemical Ecology 14:815-824

Poole JH, Payne K, Langbauer WR, Moss CJ (1988) The social contexts of some very low-frequency calls of African elephants. Behavioral Ecology and Sociobiology 22:385-392

Prop N (1960) Protection against birds and parasites in some species of tenthredinid larvae. Arch. Neerl. Zool. 13:380-447

Radford AN (2004a) Vocal coordination of group movement by green woodhoopoes (*Phoeniculus purpureus*). Ethology 110:11-20

Radford AN (2004b) Vocal mediation of foraging competition in the cooperatively breeding green woodhoopoe (*Phoeniculus purpureus*). Behavioral Ecology and Sociobiology 56:279-285

Roessingh P (1990) Chemical marker from silk of *Yponomeuta cagnagellus*. Journal of Chemical Ecology 16:2203-2216

Ruf C, Fiedler K (2000) Thermal gains through collective metabolic heat production in social caterpillars of *Eriogaster lanestris*. Naturwissenschaften 87:193-196

Seddon N, Tobias JA, Alvarez A (2002) Vocal communication in the pale-winged trumpeter (Psophia leucoptera): Repertoire, context and functional reference. Behaviour 139:1331-1359

Seymour R (1974) Convective and evaporative cooling in sawfly larvae. Journal of Insect Physiology 20:2447-2457

Stewart KJ, Harcourt AH (1994) Gorillas vocalizations during rest periods - Signals of impending departure. Behaviour 130:29-40

Tostowaryk W (1972) Effect of prey defense on functional response of *Podisus modestus* (Hemiptera Pentatomidae) to densities of sawflies *Neodiprion swainei* and *N. pratti banksianae* (Hymenoptera: Neodiprionidae). Canadian

Entomologist 104:61-69

Visscher PK, Seeley TD (2007) Coordinating a group departure: who produces the piping signals on honeybee swarms? Behavioral Ecology and Sociobiology 61:1615-1621

Vulinec K (1990) Collective security: Aggregation by insects in defense. In: Evans L, Schmidt J (eds) Insect Defenses. State University of New York Press, Albany, pp 251-288

Warkentin KJ, Keeley ATH, Hare JF (2001) Repetitive calls of juvenile Richardson's ground squirrels (*Spermophilus richardsonii*) communicate response urgency. Canadian Journal of Zoology-Revue Canadienne De Zoologie 79:569-573

Weyh R, Maschwitz U (1978) Trail substance in larvae of *Eriogaster lanestris* L. Naturwissenschaften 65:64-64

Windfelder TL (2001) Interspecific communication in mixed-species groups of tamarins: evidence from playback experiments. Animal Behaviour 61:1193-1201

CHAPTER 4

THE ART OF SOCIAL-LIVING: EXAMINING THE SELECTIVE BENEFITS OF GROUPING IN LARVAE OF A SAWFLY, *PERGA AFFINIS*

Abstract

For many organisms, enhanced defense acts as a primary selective force behind the evolution of social behavior but numerous other benefits play a role as well. Larvae of the Australian sawfly, *Perga affinis*, live in colonies and retain their gregarious lifestyle through pupation. A system of vibrational communication facilitates their gregarious habits and group-size often increases over time as colonies readily coalesce with others. To evaluate selective pressures leading to and maintaining *P. affinis*' social behavior, I investigated potential benefits of group-living. These included predation, foraging facilitation, thermoregulation, and pupation success where I compared treatments between individuals vs groups and between groups of different sizes. I found no evidence of predation; however, the mortality risk was significantly higher for single vs grouped larvae, suggesting that other grouping benefits are important. Investigating thermoregulation revealed that grouped larvae attained a significantly higher temperature excess than single individuals and that large groups reached higher temperatures than small groups. Larvae grew significantly faster at higher temperatures, indicating that the increased thermoregulatory capacity of large groups may speed up development. Group size did not affect foraging facilitation (measured via weight gain) except during the last time period, where individuals in large groups gained significantly more weight. Weight gain in the last instar may be critical for pupation as high larval weight significantly increased the odds of pupation success. Therefore, group-living offers multiple benefits for *P. affinis*, providing insight to the selective agents promoting social evolution. I discuss how diverse

selective factors often lead to convergent behaviors among social organisms.

Introduction

Questions relating to the costs and benefits of group-living provide insight as to how and why social behavior and communication evolve. As stated by Alexander (1974, p.328) "there are no automatic or universal benefits to group-living" and, in fact, the opposite may be true with "automatic and universal detriments" primarily derived from disease transmission, increased conspicuousness, and increased competition. The presence of social groups, therefore, requires some form of selective advantage to the individual, which may be assessed relative to living alone or living in different-sized groups. Additionally, the development of social behavior within groups through cooperation or communication may be seen as a means for enhancing the selective advantages (Alexander 1974).

One of the primary benefits to group-living is that of predation defense (Alexander, 1974), and this is true among insects as well (Vulinec 1990). Many gregarious insects possess chemical defenses and aposematic signals that may be enhanced by grouping (e.g., Tostowaryk 1972; Aldrich and Blum 1978; Lawrence 1990; Reader and Hochuli 2003). As grouping often increases conspicuousness, Ruxton and Sherratt (2006) argue that defense may have been an evolutionary prerequisite to the formation of aggregations in these cases. A comparison of survivorship curves between gregarious and solitary insect species with repellent defenses, indicates that gregarious species have higher larval survivorship than solitary ones (Hunter 2000). However, as the curves are shaped differently despite the fact that both have repellent defenses, the difference in survivorship is not solely due to the capacity for defense, but also stems from some additional benefits. These additional benefits have received much attention, especially in the study of larval insects where

thermoregulation (Seymour 1974; Stamp and Bowers 1990; Bryant et al. 2000), feeding facilitation and more efficient foraging (Ghent 1960 ; Lawrence 1990; Denno and Benrey 1997), growth rate (Tsubaki 1981; Breden and Wade 1987; Denno and Benrey 1997), and pupation success (Gallepp 1974; Wrona and Dixon 1991) may all be enhanced through group-living. Furthermore, the size of the group or the benefit derived from grouping may vary according to larval stage in various species (Fitzgerald 1993; Despland and Le Huu 2007). These considerations suggest that a combination of benefits, instead of a benefit coming from a single source, may drive the evolution of social behavior.

In this study I examine the potential benefits of group-living among larvae of an Australian sawfly, *Perga affinis*. *P. affinis* are members of the family Pergidae, which exhibits some of the most highly developed social behavior within the Suborder Symphyta (Order: Hymenoptera) (Smith 1993). Larvae communicate via tactile and vibrational signals (Fletcher 2007), which help to promote cohesion among group members (Fletcher 2008). With such pronounced social behavior and a system of communication to facilitate it, questions surrounding the benefits of group-living naturally arise. Like other gregarious insects, *P. affinis* larvae have an effective chemical defense that is regurgitated upon disturbance. Although the regurgitant has been shown to effectively repel ants, birds, and rodents (Morrow et al. 1976), no work has tested its effectiveness in relation to being in solitary vs group-living individuals and there has been little documentation of actual predation pressure (Carne 1969). Therefore, my first experiment addresses the question of predation and examines it in relation to grouped vs solitary larvae. The other four experiments address the additional benefits of group-living, which given the long, 6-month larval stage that occurs during the Australian winter, are likely to represent significant selective factors for social behavior.

P. affinis larvae are black and aggregate in clusters during the day, often on exposed branches. Both their dark coloration and grouping behavior during periods of solar radiation suggest a possible benefit of thermoregulation (Knapp and Casey 1986; Braby and Douglas 1992; Reavey 1993; Casey 1993; Bryant et al. 2000). To address this, my second experiment examines the degree of temperature excess (amount by which body temperature (T_b) exceeds ambient (T_a); T_b-T_a) attained by solitary vs grouped larvae as well as larvae in groups of different sizes. The third experiment tests for possible benefits of increased body temperature by examining growth rate as well as immune function at low vs high temperatures. The fourth experiment examines growth rate, but in relation to group size and time in larval development, instead of temperature. Growth rate serves as a measure of foraging efficiency, which may be enhanced by group size (e.g., Breden and Wade 1987). The experiment looks for changes in the effect of group size on growth rate over several instars, as grouping may vary in importance at different larval stages (e.g. Lawrence 1990; Fitzgerald 1993). The fifth and final experiment extends the search for group-living benefits into pupation, as larvae of *P. affinis* burrow underground *en masse* to form clusters of attached cocoons. I examine the effect of group size and the weight composition of group members on individual pupation success.

In summary, although defense is one of the most widely recognized benefits of group-living (Alexander 1974; Vulinec 1990), there are many avenues by which grouped individuals may receive selective benefits. These varied selective advantages may be important at different life-stages, having a combined effect of promoting the evolution of social living. I, therefore, examine a suite of potential benefits in a gregarious sawfly larva in order to assess their relative importance in shaping the highly-evolved social behavior in this organism.

Methods

Larvae of *Perga affinis* (Hymenoptera: Pergidae) used in all experiments came from a field site in the Australian Capital Territory, 20 km NE of Canberra, Australia. Colonies were maintained on fresh *Eucalyptus spp*. cuttings and housed in shaded enclosures prior to experimentation at the Australian National University.

Experiment 1 – Predation. To test the hypotheses that group vs solitary living confers an anti-predation and an overall survivorship advantage, I conducted a field experiment with paired groups and paired solitary larvae. Each pair consisted of an exposed and protected platform (46cm x 46cm) bordered by an 8cm high plastic fence on which the larva(e) resided for the 12-day trial period. Platforms were placed under trees, with the larvae resting on jars holding cut branches about 25-30cm off of the ground. Protected platforms differed from exposed platforms by enclosing the foliage in a 30x30cm screened box, thus preventing any contact with potential predators or parasitoids. In this way, they provided a measure of background mortality that occurred in the absence of predation. A total of 5 pairs of groups (with 20 larvae per group) and 10 pairs of single larvae were placed under *Eucalyptus spp*. trees at various locations and monitored every morning and evening for 12 consecutive days. I recorded the number of larvae present as well as their current condition (alive, sick, or dead).

The data were analyzed using the proportion test in Minitab v.15. I first looked for evidence of predation by comparing the number of larvae that disappeared or died in the exposed vs protected treatment for single individuals and groups separately. I then examined the possibility of a group-benefit by comparing the proportion of single vs grouped larvae that died or disappeared during the experiment (data on exposed and protected larvae from grouped or individual treatments were combined for the comparison).

Experiment 2 – Group Size and Thermoregulation. I examined the thermoregulatory and heat retention capacity of larvae by comparing the temperature difference between their bodies and the ambient air in shaded and sunny conditions. In addition, I examined this temperature difference in relation to group size by collecting data on single larvae, small groups (average $n = 4.3 \pm 0.82$) and large groups (average $n = 17.5 \pm 3.13$). Larval measurements were taken by placing a T-type hypodermic micro thermocouple probe on the ventral surface of an individual; I took three measurements per larva or group and used the mean value for the analysis.

All larvae and groups sat on exposed *Eucalyptus spp*. branches in either shaded or sunny conditions at least 15 minutes prior to taking measurements. Under shady conditions, I examined 5 large groups of larvae and 12 solitary individuals. Under sunny conditions, I collected data on 16 large groups, 6 small groups and 13 solitary individuals. Recordings were made on clear days between noon and 14:00.

Data analysis involved non-parametric statistics using Minitab v.15. For shaded conditions, I used the Wilcoxon signed rank test to compare the difference between larval body temperature and ambient temperature against the null of zero; the Mann-Whitney test provided a comparison of this temperature difference between grouped vs single larvae. For sunny conditions, I used the Kruskall-Wallis test to compare the difference between body vs ambient temperature for individuals, small groups and large groups. The Mann-Whitney test with a corrected alpha value of 0.0167 was used for post-hoc pairwise comparisons between the three categories.

Experiment 3 – Temperature: Effects on Growth Rate and Immune Function To test the effect(s) of temperature on growth rate and immune function in *P. affinis* larvae, I placed both groups and individuals under warm and cold temperature conditions in environmental chambers for a period of two weeks. Working with grouped and single larvae allowed me to determine if growth was a function of larval

social context, ambient temperature, or their interaction. A total of 3 groups (20 larvae per group) and 20 individuals were placed in each temperature treatment; all larvae resided on *Eucalyptus spp*. cuttings from the same host tree. Both chambers were on an 11:13 light:dark cycle, with the day and night temperatures set at 24.2 °C and 6 °C for the warm treatment and 11 °C and 6 °C for the cold treatment, respectively.

To measure larval growth rate, I weighed larvae at the start and end of the experiment. In order to record the weight change of individuals within a group, I randomly chose and marked 5-7 larvae per group with colored paint. Data were analyzed in Minitab v.15 using a General Linear Model with weight gain as the dependent variable and larval social context (Grp/Ind), temperature (W/C), and their interaction as the predictors.

Testing for immune function involved examining the effect of larval hemolymph on the growth of *Escherichia coli* bacteria, strain K12-LS3. Using a capillary tube, I collected 1 µl of hemolymph from 20 single larvae and 20 grouped larvae, half of which came from the warm treatment and half from the cold treatment. In a 96-well microtiter plate, I added 250 µl of media (minimal salts media and vitamin B_1, Schlegel 1992) to each well followed by 1 µl of hemolymph and 10 µl of an inoculated solution of *E. coli* at a density of 2.0×10^6 cells/ml. Control wells had everything added to them but the hemolymph. To ensure that the concentration of cells in the hemolymph was the same for warm vs cold larvae, I used a hemocytometer to conduct a cell count on 1µl of hemolymph from 4 warm and 4 cold larvae. For each individual, I counted 5 squares within the large center square and divided the total by the volume of fluid in the 5 squares to calculate the cell concentration per µl. Cell concentrations for the two treatments were compared using a Kruskall-Wallis test.

The microtiter plate was placed in a Powerwave 10 microplate scanning

spectrophotometer (Bio-tek Instruments, Inc.) where changes in the turbidity of the cultures (640 nm) were monitored every 5 minutes. The micro-titre plate was shaken at maximum speed for one minute prior to each reading, and data were collected until absorbance readings had attained a maximum plateau. Absorbance values (recorded as optical density (OD)) provided a measure of light transmittance, where a high OD corresponded to low light transmittance through the media. The level of light transmittance relates to the density of *E. coli* and hence, OD provides a measure of the amount of *E.coli* in a given well. Data were analyzed with SPSS v.15 by running a General Linear Model with max OD as the dependent variable and larval social context, temperature, and their interaction as the predictors.

Experiment 4 - Group Size and Feeding Efficiency. To examine how group size affects feeding efficiency in larval colonies, I measured the growth rate of individuals across several instars. All colonies resided in a shadehouse, thereby eliminating any unequal thermoregulatory benefits of direct solar radiation while ensuring that the groups were kept at the same ambient temperatures. I conducted the study over two field seasons. In the first season of 2003, I monitored larvae from mid-July through to pupation in mid-September, covering the 4^{th}, 5^{th}, and 6^{th} instars. The second season in 2004 covered the early instars of 2, 3, and some 4, as larvae were monitored from late May to mid-July. I followed a similar experimental procedure for both field seasons; any differences have been noted below.

In 2003, I collected 7 field colonies that were then separated into 3 groups based on weight. The light group contained individuals weighing 0.2-0.3g, the medium group had larvae weighing 0.31-0.39g, and the heavy group had larvae weighing 0.4g and higher. I divided larvae into 6 large and 6 small experimental colonies, containing 20 and 5 individuals, respectively. Each large experimental colony received 6 light, 8 medium, and 6 heavy larvae while each small experimental

colony received 1 light, 2 medium, and 2 heavy larvae. In 2004, I collected several colonies from 6 different host trees, creating the 6 large and 6 small experimental colonies with larvae from the same host tree. This allowed me to test for any effects of host tree on larval growth. During the experiment I maintained larval numbers between 15-20 for the large groups and 3-5 for the small groups by adding additional individuals when original members died. Any larvae added to the 2004 experimental colonies were collected from the original host tree. Five to six larvae per colony were individually marked with enamel paint on the back of their head capsule and again on the sclerotized uropod of the abdomen for the purposes of tracking individual growth rates. If a larva molted without being seen and repainted, I simply painted a different colony member so that I could maintain about 5-6 marked individuals per group.

Experimental colonies resided on cut *Eucalyptus spp*. branches (all from the same tree in 2003 or from their original host tree in 2004) that were placed in bottles of water in an outdoor shadehouse. To avoid any positional effects within the shadehouse, the positions of the bottles were rotated every two weeks. Measurements of the larval weight and head capsule width were taken each fortnight in 2003 and weekly in 2004; colonies were monitored daily for molting activity.

Data were analyzed the data in SPSS v.15 using a Mixed Model Analysis with weight change (mg/day) as the dependent variable. Weight change was measured for individual larvae over each time period interval during the experiment (an interval comprised two weeks in 2003 and one week in 2004). The fixed predictor variables were time interval (1-9 in 2004; 10-15 in 2003), group size (L/S), the interaction between time interval and group size, and two binary predictors related to molting: 1) molted during the given time interval and 2) molted during the previous time interval. The two random predictors were colony and larva; data from 2004 had host tree as a third random predictor. *Post-hoc* pairwise comparisons of the main effects included

the appropriate Bonferroni adjustments for multiple comparisons.

Experiment 5 – Pupation Success: Group Size and Group Composition. Upon entering the soil for pupation, *P. affinis* may reside underground from a few months to several years before emerging as an adult (Carne, 1962). Due to this variability, I measured pupation success in the experiment by checking for pre-pupal viability at least 3 weeks after burial underground.

The experiment was conducted in two parts. The first examined the effect of group size on pupation success by looking at the proportion of larvae from large and small colonies that developed into viable pre-pupae. I had a total of 10 large colonies ranging in size from 14-38 individuals and 11 small colonies ranging from 2-7 individuals per group. In mid-September, water bottles holding the foliage and colonies were placed inside uncovered styrofoam boxes (53.5 x 25 x 28cm tall for the large groups and 23 x 15.5 x 15.2cm tall for the small groups) filled to a depth of 12 cm with soil. This set-up provided suitable burial habitat for each colony once they were ready to descend the branches for pupation. Upon excavating the cocoons, I checked for viability of the pre-pupa within by using a scalpel and dissecting microscope to make a small incision for detecting movement by the individual. The incision was then patched with small pieces of mulberry paper and rice paste. I compared the proportion of pre-pupa per group that was alive between large and small colonies using a Mann-Whitney test.

The second part of the experiment examined two additional factors that could affect pupation success: 1) the weight of a larva directly prior to pupation and 2) the weight composition of larvae within an individual's colony. All larvae for the study were collected from a total of 5 field colonies, each of which contained both small (less than or equal to 1.0g) and large (greater than 1.3g) individuals. Collecting from such colonies ensured that the weight extremes used in the study accurately

represented the extent of variation seen in nature. I mixed together larvae from the 5 colonies and then separated them based on weight for placement into the three treatment groups. Treatments consisted of three different weight compositions for the experimental colonies: colonies with large (L) larvae (> 1.3g), colonies with half-large and half-small (LS) larvae, and colonies with small (S) larvae (≤ 1.0g). I had a total of 15 colonies providing 5 for each treatment. Each experimental colony had a total of six larvae that were individually marked with enamel paint on the head capsule and the tip of the abdomen for identification.

Experimental colonies resided in separate containers consisting of a 23cm x 15.5cm x 15.2cm styrofoam box filled with dirt holding a bottle of fresh foliage. I monitored colonies daily for the location and time of burial and took weekly measurements of larval weight. As before, pupation success was measured by excavating the cocoons and checking for pre-pupal viability. Because the larvae shed their cuticle within the top chamber of the cocoon, I was able to identify individual pre-pupae based on the colored markings on the shed cuticle. Data were analyzed in Stata v.10 using a binary linear regression (xtmelogit) with the dependent variable being whether or not an individual was alive as a pre-pupa. The predictors were group weight composition (L, LS, S), weight designation of larva (S or L), and the larva's final weight before pupation. The two random factors were colony and larva.

Results

Experiment 1 – Predation. To look for evidence of predation, I first compared the proportion of larvae that disappeared or died in the exposed vs protected treatment for single individuals and groups separately. For both individuals and groups there was no difference in the proportion that disappeared or died between the exposed and protected treatments (Proportion test, $Z = 0.93$, $P = 0.351$; $Z = 1.37$, $P = 0.172$,

respectively). This fails to provide evidence of predation because the mortality in the exposed treatment is equivalent to the expected background mortality for the given social context. When comparing mortality between grouped vs single individuals (combining data from the exposed and protected treatments), single larvae had a significantly higher proportion of individuals disappear or die than grouped larvae at 40% vs 7%, respectively (Figure 4.1; Proportion test, $Z = -2.92$, $P = 0.003$), suggesting that grouping itself provided a survival advantage.

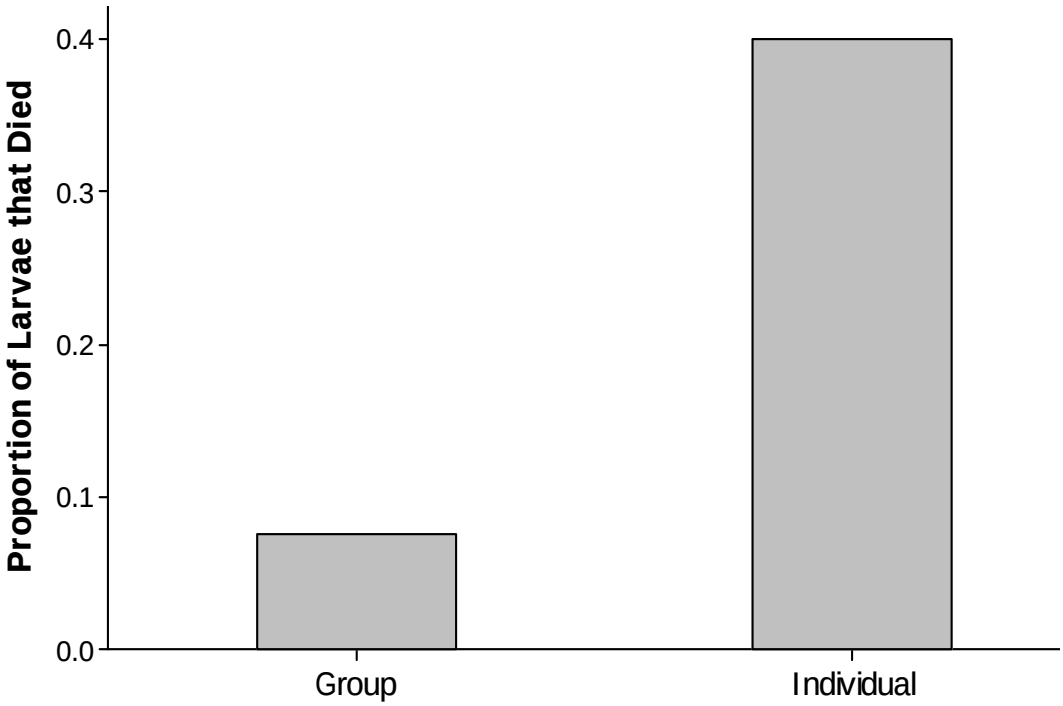


Figure 4.1 Proportion of larvae that died in the grouped vs individual treatment. A significantly higher proportion of larvae from the individual treatment died than from the grouped treatment (Proportion test $Z = -2.92$, $p = 0.003$).

Experiment 2 – Group Size and Thermoregulation. Under shaded conditions, there was no significant difference between the body temperature of grouped vs single larvae (Mann-Whitney test, $W = 39$, $P = 0.9512$). Therefore, I combined data on groups and single larvae for an overall comparison between larval body temperature

and ambient temperature under shaded conditions. Body temperature was significantly higher than ambient, at a median of 0.7°C above, suggesting a slight ability for larvae to regulate body temperature in the absence of direct solar radiation (Wilcoxon signed rank test, $W = 133$, $P = 0.001$).

Under sunny conditions and regardless of social context, larval body temperature was significantly higher than ambient, at a mean of 10°C above, indicating an ability to retain solar heat (Paired *t*-test, $t_{33} = -16.84$, $p < 0.0001$). When considering social context (large and small groups, single larvae), there was a significant difference in the ability of larvae to retain heat and attain a temperature excess above ambient in large vs small groups as well as individuals (see Figure 4.2; Kruskall-Wallis $H_2 = 22.29$, $P < 0.0001$). Pairwise comparisons indicate that large groups retained significantly more heat than small groups (Mann-Whitney $W = 220$, $P = 0.0044$) and that both large and small groups retained more heat than single larvae (Mann-Whitney: large group vs singles $W = 333$, $P < 0.0001$; small group vs singles $W = 97$, $P = 0.0014$).

Experiment 3 – Temperature Effects on Growth Rate and Immune Function. After controlling for social context (Grp vs Ind), temperature had a significant effect on growth rate with larvae under the warm treatment gaining significantly more weight than those under cold conditions (Figure 4.3a; GLM, $F_{1,61} = 31.77$, $P < 0.0001$). Under both temperature conditions, grouped larvae gained more weight than lone individuals but the difference was not significant (GLM, $F_{1,61} = 2.36$, $P = 0.13$). The results suggest that given the benefit of increased body temperature (in this case, through a warm ambient temperature) single larvae perform just as well as grouped larvae in terms of weight gain. Similarly, under cold ambient temperatures (with no source of solar heat), there is no extra benefit of being in a group.

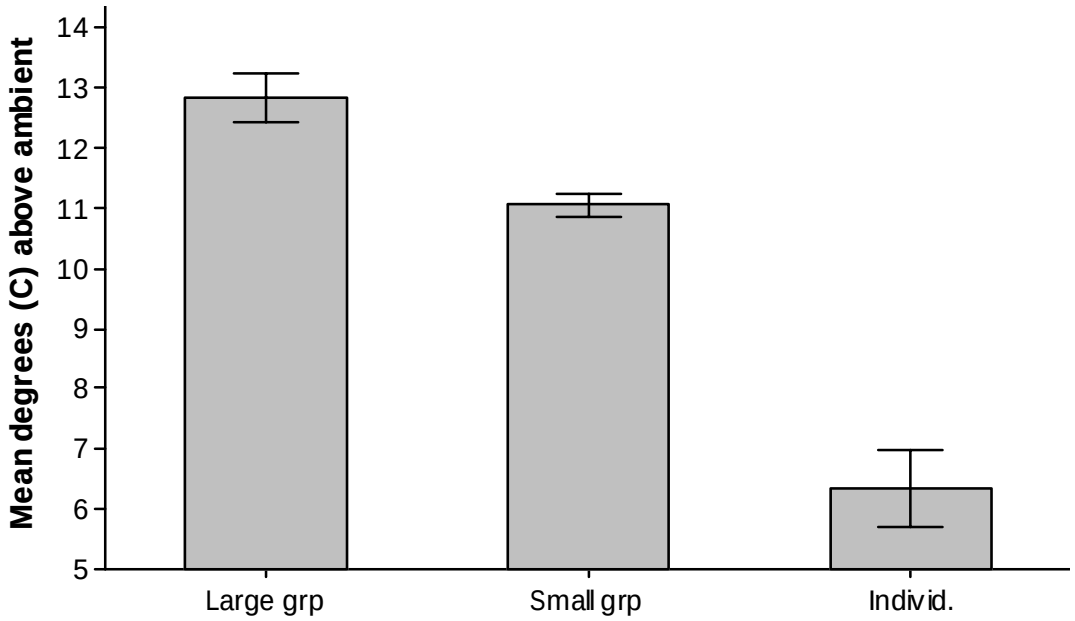


Figure 4.2 A comparison of heat retention by larvae in different social contexts. Under sunny conditions, larvae in groups retained significantly more heat than lone individuals and larvae in large groups retained significantly more heat than those in small groups, thereby reaching a higher body temperature (T_b) (Kruskall-Wallis $H_2 = 22.29$, $p<0.0001$). Error bars are ± 1 SD.

When examining immune function, temperature treatment had a significant effect on the ability of larval hemolymph to limit bacterial growth (GLM, $F_{1,31} = 32.52$, $P < 0.0001$); social context had no effect ($F_{1,31} = 0.519$, $P = 0.477$). The maximum optical density (OD) for wells that received 'warm' hemolymph was significantly higher than the OD for those that received 'cold' hemolymph (Figure 4.3b). As a high OD indicates low light transmittance and hence a larger population of *E. coli*, the 'warm' hemolymph was less effective in inhibiting bacterial growth than

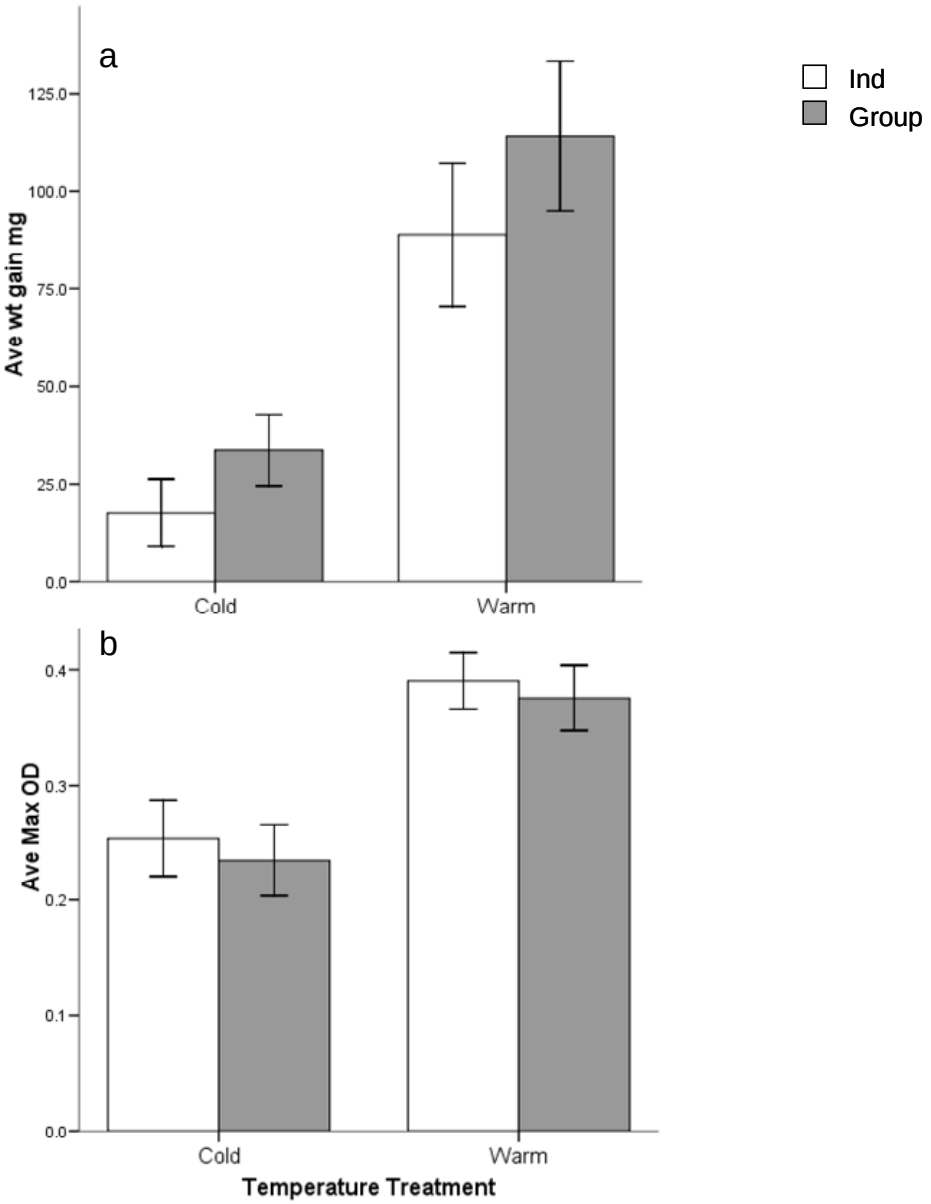


Figure 4.3 a. Average weight gain for grouped vs individual larvae under cold and warm temperature treatments. Both grouped and individual larvae grew significantly more under the warm than cold treatment but social context had no effect on growth within a given temperature treatment (GLM for C/W: $F_{1,61} = 31.77$, $p < 0.0001$). b. Maximum optical density (OD) for bacterial cultures treated with 'cold' vs 'warm' hemolymph. As high OD values relate to large populations of bacteria, cultures treated with 'warm' hemolymph had significantly higher densities of *E.coli* than those treated with 'cold' hemolymph, suggesting a difference in immune function between the two treatments (GLM for C/W: $F_{1,31} = 32.52$, $p < 0.0001$). Social context had no effect on OD. Error bars are ± 1 SD.

the 'cold' hemolymph. A comparison of cell concentration in hemolymph from cold vs warm larvae showed that hemolymph from cold larvae had a significantly higher cell count than that of warm larvae, at 9.4 x 10^6 vs 7.2 x 10^6 cell/µl, respectively (Kruskall-Wallis $H_1 = 5.4$, $P = 0.02$). Cold larvae also weighed significantly less than the warm individuals, suggesting that their hemolymph may have been more concentrated (Figure 4.4; Kruskal-Wallis $H_1 = 5.33$, $P = 0.02$). This higher cell concentration may account for the increased immune function of the hemolymph from the cold-reared larvae.

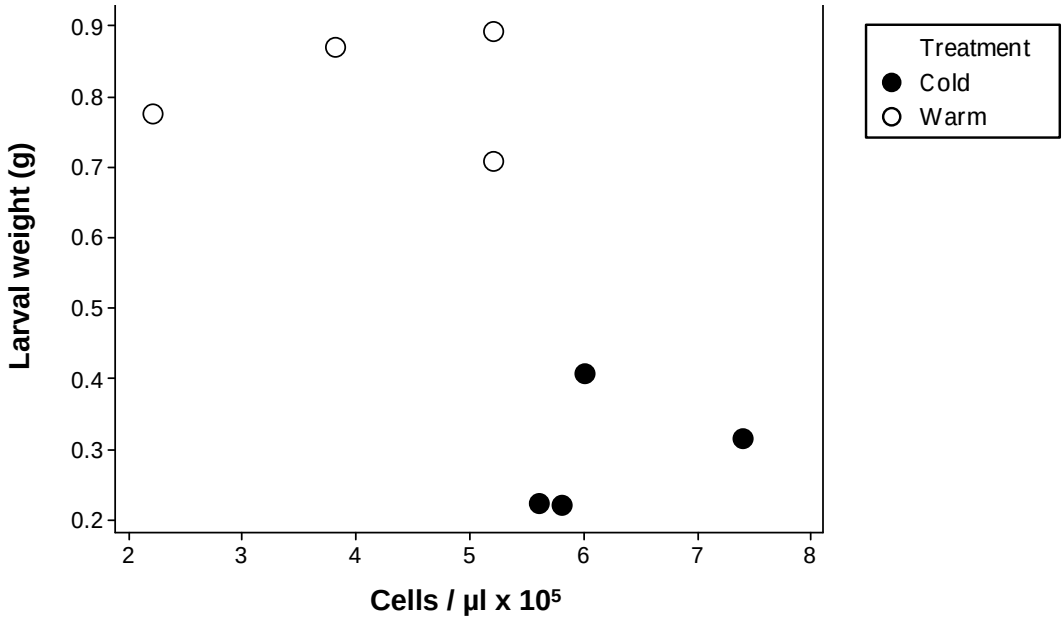


Figure 4.4 Relationship between larval weight and hemolymph cell concentration. Lighter larvae tended to have higher cell concentrations than heavier individuals. Open circles represent larvae from the warm treatment while closed circles represent larvae from the cold treatment.

Experiment 4 – Group Size and Feeding Efficiency. In 2003, I recorded growth rate, which served as a measure of feeding efficiency for 4th, 5th, and 6th instars that were weighed at biweekly intervals from mid-July until pupation. Colony size, time interval, and the interaction between them were significant predictors in the model

(Mixed Model $F_{1,61} = 7.28$, $p = 0.009$; $F_{4,161} = 55.97$, $P < 0.0001$; $F_{4,158} = 3.5$, $P = 0.009$, respectively), indicating that growth rate varied over time and that the variation depended on group size. As shown in Figure 4.5, both large and small colonies had similar growth rates during intervals 11 and 12; however, at interval 13, the rates diverged with larvae from large colonies growing slightly faster than those from small colonies. By the last time interval before pupation, the difference in growth rate was significant, with larvae from large colonies growing at a rate of 40.5 ± 2.15 mg/day vs 26.7 ± 2.37 mg/day for individuals in small colonies (Paired *t*-test $t_{50} = 3.547$, $P = 0.001$). Another significant predictor in the model was whether or not an individual had molted during the previous time interval. Larval growth rate was significantly higher during time intervals that followed a molt, with a mean increase of 5.8 ± 1.74 mg/day relative to other times (Mixed Model $F_{1,182} = 11.23$, $P = 0.001$).

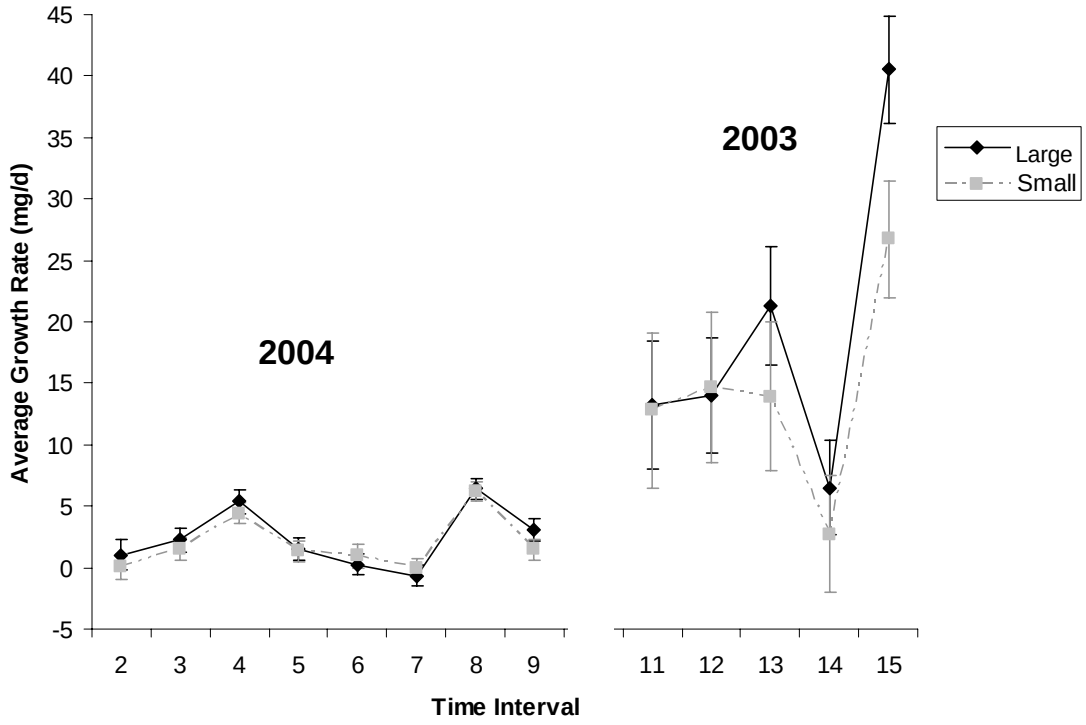


Figure 4.5 Average growth rate of larvae in large vs small colonies across time intervals. Larvae in large colonies grew significantly faster than those in small groups during the last time interval (Bonferroni-adjusted Paired *t*-test $t_{50} = 3.547$, $p = 0.001$). Error bars are ± 2 SE.

In 2004, I worked with early instars, monitoring their growth rate at weekly, instead of biweekly, intervals until mid-July. This allowed for closer monitoring of growth rate over time as well as its association with the molting cycle. As seen in 2003 with the later instars, time interval and the interaction between time interval and group size were significant predictors in the model (Mixed Model $F_{8,256} = 53.81$, $P < 0.0001$; $F_{7,320} = 2.28$, $P = 0.028$, respectively); however, group size alone had no significant effect on growth rate (Mixed Model $F_{1,4} = 1.8$, $P = 0.245$). Larvae from both large and small colonies followed the same cyclic pattern in growth rate over time (Figure 4.5). Although at times the lines are not parallel, which explains the significance in the interaction term, there was no point at which growth rate differed significantly between the large and small colonies. The two binary predictors related to molting had a significant effect on growth rate, where overall growth increased by 1.9 ± 0.27 mg/day during time intervals following a molt and decreased by 0.94 ± 0.28 mg/day during time intervals in which molting occurred (Mixed Model $F_{1,321} = 48.53$, $P < 0.0001$ and $F_{1,321} = 11.4$, $P = 0.001$, respectively). The effect of molting on growth rate may explain the cyclic pattern of peaks and valleys, where the peaks occur after molting and the valleys occur prior to and at the time of molting.

Experiment 5 – Pupation Success: Group Size and Group Composition. Pupation success was measured by examining the viability of pre-pupae after they had burrowed underground and spun a cocoon. Group size had no effect on the proportion of individuals that were alive as pre-pupae. Large colonies (≥ 14 individuals) had a median proportion of 0.89 ± 0.028 pre-pupae that were alive, while small colonies (≤ 7 individuals) had a median of 0.75 ± 0.1 (Mann-Whitney test $W = 120$, $P = 0.4953$).

The second part of the experiment examined how weight prior to pupation as well as the weight composition of colony members affected an individual's pupation success. The odds of being alive as a pre-pupa were significantly affected by a larva's

weight prior to pupation, increasing by a factor of 322 for every unit change in weight (Figure 4.6; Binary logistic regression $Z = 3.98$, $P < 0.0001$). Neither the weight composition of the group nor a larva's original designation as being S or L at the start of the study had a significant effect on pupation success.

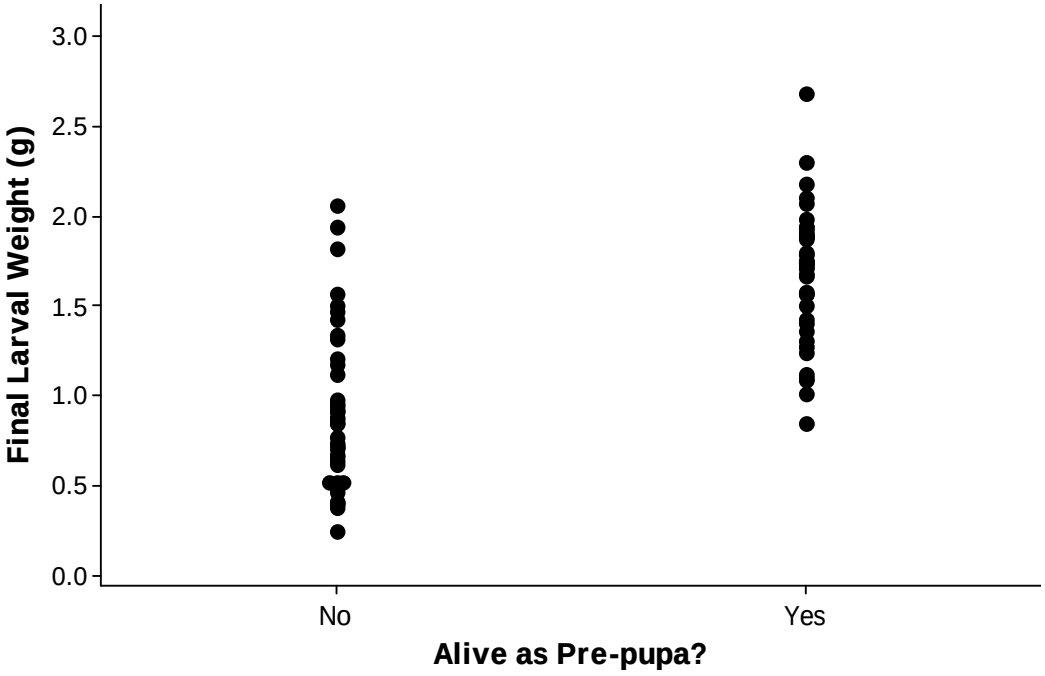


Figure 4.6 Pre-pupal viability and larval weight prior to entering diapause. Larval weight significantly affected the odds of pre-pupal viability, with heavier larvae having higher survivorship than lighter ones (Binary logistic regression $Z = 3.98$, $p < 0.0001$).

Discussion

Experiments 1, 4, 5 – Predation, Foraging & Group Size, Pupation Success.

Although defense is one of the most widely recognized benefits of group-living, I found no evidence of predation on *P. affinis* larvae. Predation may occur during the very early instars, as this would coincide with late autumn when arthropod predators may still be active, but overall, the results corroborate previous work by Carne (1969)

where the major sources of mortality stemmed, not from predation, but from other factors such as desiccation, fungal disease, and parasitism.

The lack of predation, and hence, predation pressure on *P. affinis* larvae is surprising given the classic defensive attributes of the colonies. Larvae form conspicuous clusters on exposed branches during the day, have an effective chemical defense (Morrow et al. 1977), and disperse to feed under the protective cloak of night. Many insects, especially larvae, have adopted nocturnal feeding along with other strategies that likely evolved in response to predation pressure. Downes (1987) argues convincingly that several primary features of extant arthropods, including nocturnal feeding, the separation of larval and adult life, and chemical defenses, evolved in response to early terrestrial vertebrate predation in the Carboniferous period. While early vertebrates may have played a significant role in the diversification of arthropod lineage, their impact on present day fauna is variable (Codella and Raffa 1993). I believe this underscores the importance of including a historical, along with a present-day, perspective when seeking to understand the suite of traits in an organism. Whitehouse and Lubin (2005, p. 352) share a similar view by stating that "the proximate function that drives group traits is not necessarily the function that selected for group formation in the first place." Predation no doubt played a role in the evolution of pergid social behavior, but its role may have been historical relative to current selective pressures that now provide the major adaptive advantages for a gregarious life-style.

The significant difference in survivorship between lone and grouped larvae in the first experiment (Figure 4.1) serves to reinforce the fact that other selective pressures play a role in shaping the gregarious behavior. Several studies have documented poor survivorship of lone vs grouped larvae (Ghent 1960; Lyons 1962; Carne 1969; Fitzgerald and Visscher1996) and, in some cases, this has been attributed

to lack of feeding facilitation in the early instars. However, most of these species remain in groups well beyond the first two instars, suggesting that feeding facilitation is not the sole benefit. Kalin and Knerer (1977) pointed to potential 'psychological' factors as a cause for higher mortality, noting that separated larvae of the gregarious sawfly *Neodiprion leconti* fed and inflicted more incisions in the foliage than when grouped, but that they appeared restless, spending more time wandering in between feeding bouts. Similarly, Clayton's (1978, p. 375) review on socially facilitated behavior, states that "isolation of a normally social animal leads to the inhibition of many activities," mainly due to the stress of being alone. This may indeed be the case with *P. affinis*, as lone larvae often appear agitated, tapping their abdomens on the substrate with higher frequency than grouped individuals (Fletcher 2007). Likewise, the act of feeding may be socially facilitated through both tactile and vibrational communication signals of neighboring group members (Fletcher 2007), such that isolated individuals may feed less due to the lack of stimulation.

Given that the presence or absence of conspecifics affects the survivorship of *P. affinis* larvae, and that this may stem from feeding differences, Experiment 4 sought to determine whether or not group-size affects foraging efficiency, which was measured via growth rate. The experiment took place over several instars, allowing for the examination of a group-size effect at different larval stages, as it has been shown to be most important in the early instars of several species (Ghent 1960; Lyons 1962; Denno and Benrey 1997; Fordyce 2003). After controlling for a larva's stage in the molting cycle, group size only had a significant effect on growth rate during the last instar, about two weeks prior to pupation (Figure 4.5). This matches the natural history of *P. affinis* where colonies readily merge upon meeting on the host tree; by the end of the season, larvae reside in huge groups of up to several hundred individuals (Carne 1962; Fletcher pers obs) and such massive aggregations only

appear close to the time of descending the tree for pupation. Interestingly, a similar result occurred in a liparid caterpillar, *Euproctes phaeorrhaea*, where the growth rate of grouped larvae was greater than that of solitary ones during the period prior to entering diapause (Grison 1948). In both cases, the mechanism for the greater growth rate is not known, although a study by Carne (1962) documented a very rapid increase in the food consumption, as measured by fecal pellet weight in the 5th and particularly the 6th instar of *P. affinis*. Perhaps such an increase is socially facilitated, being significantly more pronounced in larger groups.

Results from the second pupation study in Experiment 5 also provided indirect support for the idea that large groups offer greater benefits, especially prior to diapause. Higher larval weight during the final instar significantly increased the odds of pre-pupal vitality (my measure of pupation success). Larval weight also correlates with pre-pupal weight, which has a significant effect on the fecundity of females (Carne 1969). Therefore, grouping in general provides survivorship benefits to larvae and the size of the group may be particularly important during the final instar, as it facilitates rapid growth rates and heavier pre-pupae. These results offer an empirical example of ontogenetic changes in grouping, due to colony merging, that coincide with a specific adaptive benefit of gregarious living.

Experiments 2, 3 – Thermoregulation, Growth Rate, Immune Function. While foraging and the social facilitation of foraging directly impact larval development and growth, temperature also plays an important role. As ectotherms, larvae may rely on behavioral and/or phenotypic strategies to gain some degree of thermoregulatory ability. Body orientation and postural adjustments can maximize the surface area exposed to solar radiation, and forming a group serves to increase an individual's effective body size, which, in turn, increases the maximum temperature excess (the amount by which $T_b > T_a$) that it can attain (Casey 1993; Reavey 1993). Dark

coloration enhances radiant heating (Casey 1993), and the arrangement of setae can provide insulation to reduce convective heat loss without reducing radiant heat gain (Casey and Hagel 1981). Larvae of *P. affinis* have very short setae that are unlikely to aid in thermoregulation, but their cuticle is black and their aggregative behavior directly impacts the T_b of larvae when in solar radiation. Groups reached a significantly higher temperature excess than individual larvae under similar sunny conditions, a finding that corroborates Seymour's (1974) work on *Perga dorsalis*, although he used dead larvae. Group size also had a significant effect, with large groups reaching a higher temperature excess than small groups.

Experiment 3 demonstrated a clear effect of temperature on growth rate with both single and grouped larvae growing significantly more under warm than cold ambient temperature regimes. As the experiment occurred in growth chambers with no access to solar radiation, the lack of difference in growth rate between solitary and grouped larvae provides further evidence that any thermoregulatory advantage of being in a group stems from an enhanced capacity to retain solar radiation. Given that *P. affinis* larvae are active in the winter when the ambient temperatures are low, a greater ability to capture radiant heat and reach a higher temperature excess may be critical to growth. This is true for early spring larvae in northern temperate areas where the ambient temperature often does not exceed 5°C (Knapp and Casey 1986). The added advantage of larger groups reaching higher temperature excesses than smaller groups may also be important as completing development earlier allows larvae to burrow underground before the soil hardens and parasites emerge, both of which are major causes of mortality (Carne 1969).

Thermoregulation and the resultant increase in body temperature not only promote growth but can also enhance immune function as seen in organisms that induce behavioral fevers (Carruthers 1992; Karban 1998; Ouedraogo 2004). This may

not be the case in *P. affinis*, but the results are inconclusive. Testing the hemolymph from larvae in warm vs cold treatments showed a significant difference in the ability to inhibit bacterial growth; however, contrary to expectations, the cold hemolymph had higher immune function. This is likely due to the higher concentration of cells in the cold hemolymph, which may stem from the smaller size of the larvae. A more definitive test of this potential benefit and role of thermoregulation would involve inoculating larvae with a bacterial infection and comparing survivorship across different temperature regimes.

Benefits of Group-Living and the Evolution of Social Behavior. In summary, results from these experiments suggest that *P. affinis* larvae gain a survivorship advantage through group-living and that this advantage stems from both thermoregulatory and social feeding facilitation benefits. Additionally, each benefit is enhanced by an increase in group-size. A group-size advantage is also evident when considering the three major sources of mortality for *P. affinis* larvae, which are parasitism, fungal disease, and desiccation (Carne 1969). Parasitism often occurs as larvae descend trees *en masse* for pupation, so that a larger group provides an advantage through the dilution effect (Hamilton 1971). This is true for fungal disease as well, which occurs in water-logged cocoons where those that get wet provide a shield for others in the group. Likewise, desiccation takes place as larvae attempt to burrow in hard soils; having a larger number of digging individuals statistically increases the likelihood of at least one succeeding, thereby providing a route for others to get underground.

Thus, group-living in *P. affinis* provides tangible benefits, providing an explanation as to why they have evolved an effective communication system that facilitates cohesion among colony members (Fletcher 2008). Given their membership in Symphyta, a primitive suborder of Hymenoptera, and their ancestral phylogenetic

relationship to eusocial insects, one might postulate that their behavior provides insight into the evolution of eusociality. Eusociality is characterized by having cooperative brood care, an overlap of generations, and a reproductive division of labor (Wilson 1971). It is a very specific type of sociality where the function of the group centers on reproduction. In contrast, the primary function(s) of most larval societies is one of foraging and/or defense. Despite these functional differences, larval social behavior may have originated in a similar manner, i.e. through family units with high relatedness where indirect fitness benefits would offset any costs to grouping. Oviposition patterns of females through the clustering of eggs likely facilitated the evolution of the first larval social groups among siblings (Costa and Pierce 1997). Similarly, monogamy, and hence, high-relatedness, is the ancestral state for all 8 independent lineages of eusocial insects (Hughes et al. 2008), suggesting that kin selection was important in the development of this form of sociality.

Their evolutionary pathways to sociality likely diverge here, however, due to the different functions of foraging/protective groups vs reproductive groups. Many larval societies that start out as sibling colonies later merge with others, leading to low intra-group relatedness (Carne 1962; Costa and Ross 1993, 2003). The fact that these colonies continue to function as cohesive units despite the lowered relatedness, suggests that grouping behavior provides other benefits that do not rely on kin selection. In a study on tent caterpillars (*Malacosoma americanum*), colony-mixing had no effect on larval fitness but group size had a significant effect on growth rate and therefore, the final weight achieved by the larvae (Costa and Ross 2003). As final larval weight is highly correlated with adult reproductive success (Stamp and Casey 1993), it suggests that grouping, regardless of relatedness between members, provides inherent growth benefits that directly affect an individual's fitness. This may be true in *P. affinis* as well given that group size affects both thermoregulatory capacities of

larvae as well as growth rate during the final instar. It should be noted that many eusocial species have low colony relatedness due to multiply-mated queens, but in all cases this is a derived condition occurring after the evolution of anatomical caste-differentiation (Hughes et al. 2008). Here, eusociality has reached a "point of no return" and the benefits of grouping are primarily derived through group selection, where reproduction again is key (Hughes et al. 2008; Wilson and Hölldobler 2005).

In conclusion, when examining factors related to the evolution of sociality it becomes clear that the selective influences are numerous. They vary across evolutionary time, as seen in the role of predation pressure and kin selection in group formation (Downes 1987; Hughes et al. 2008), and across the life span of an individual. They also vary in the selected function of grouping, leading to primarily foraging, protective, or reproductive units (Whitehouse and Lubin 2005). While the mechanisms leading to sociality may differ, the results are often convergent. Thus, despite the phylogenetic distance between *P. affinis* and its eusocial relatives, or even the taxonomic jump to social mammals, all of them share common characteristics. Wilson (1971, p.6) demonstrates this by his single, essential criterion for sociality: "reciprocal communication of a cooperative nature." Regardless of a group's function, communication is key as it provides the means of group formation and maintenance, thereby making all other benefits possible. In fact, in his treatise on the evolution of social behavior, Alexander (1974) argues that social behavior evolved to enhance the benefits of group-living. *P. affinis*, with its effective system of communication, is able to capitalize on the benefits of group-living, a life-style shaped by multiple selective 'sculptors'.

REFERENCES

Aldrich JR, Blum MS (1978) Aposematic aggregation of a bug (Hemiptera: Coreidae) - Defensive display and formation of aggregations. Biotropica 10:58-61

Alexander R (1974) The evolution of social behavior. Annual Review of Ecology and Systematics 5:325-383

Braby MF, Douglas F (1992) Observations on the biology of *Delias harpalyce* (Donovan) (Lepidoptera: Pieridae) near Melbourne, Victoria. Australian Entomological Magazine 19:9-18

Breden F, Wade MJ (1987) An experimental study of the effect of group size on larval growth and survivorship in the imported willow leaf beetle, *Plagiodera versicolora* (Coleoptera, Chrysomelidae). Environmental Entomology 16:1082-1086

Bryant S, Thomas C, Bale J (2000) Thermal ecology of gregarious and solitary nettle-feeding nymphalid butterfly larvae. Oecologia 122:1-10

Carne P (1962) The characteristics and behaviour of the sawfly *Perga affinis affinis* (Hymenoptera). Australian Journal of Zoology 10:1-34

Carne P (1969) On the population dynamics of the eucalypt-defoliating sawfly *Perga affinis affinis* Kirby (Hymenoptera). Australian Journal of Zoology 17:113-141

Carruthers RI, Larkin TS, Firstencel H, Feng ZD (1992) Influence of thermal ecology on the mycosis of a rangeland grasshopper. Ecology 73:190-204

Casey T (1993) Effects of temperature on foraging of caterpillars. In: Stamp N, Casey T (eds) Caterpillars: Ecological and evolutionary constraints on foraging. Chapman and Hall, New York, pp 5-28

Casey T, Hegel J (1981) Caterpillar setae: Insulation for an ectotherm. Science 214:1131-1133

Clayton DA (1978) Socially facilitated behavior. Quarterly Review of Biology 53:373-392

Codella SG, Raffa KF (1993) Defense strategies of folivorous sawflies. In: Wagner M, Raffa KF (eds) Sawfly life history and adaptations to woody plants. Academic Press, Inc., San Diego, pp 261-294

Costa J, Pierce N (1997) Social evolution in the Lepidoptera: ecological context and communication in larval societies. In: Choe J, Crespo B (eds) The evolution of social behavior in insects and arachnids. Cambridge University Press,

Cambridge, UK, pp 407-442

Costa JT, Ross KG (1993) Seasonal decline in intracolony genetic relatedness in Eastern tent caterpillars - Implications for social evolution. Behavioral Ecology and Sociobiology 32:47-54

Costa JT, Ross KG (2003) Fitness effects of group merging in a social insect. Proceedings of the Royal Society B-Biological Sciences 270:1697-1702

Denno RF, Benrey B (1997) Aggregation facilitates larval growth in the neotropical nymphalid butterfly *Chlosyne janais*. Ecological Entomology 22:133-141

Despland E, Le Huu A (2007) Pros and cons of group living in the forest tent caterpillar: separating the roles of silk and of grouping. Entomologia Experimentalis Et Applicata 122:181-189

Downes WL (1987) The impact of vertebrate predators on early arthropod evolution. Proceedings of the Entomological Society of Washington 89:389-406

Fitzgerald TD (1993) Sociality in caterpillars. In: Stamp N, Casey T (eds) Caterpillars: Ecological and Evolutionary Constraints on Foraging. Chapman and Hall, New York, pp 372-403

Fitzgerald TD, Visscher CR (1996) Foraging behavior and growth of isolated larvae of a social caterpillar, *Malacosoma americanum*. Entomologia Experimentalis et Applicata 81:293-299

Fletcher LE (2007) Vibrational signals in a gregarious sawfly larva (*Perga affinis*): Group coordination or competitive signaling? Behavioral Ecology and Sociobiology 61:1809-1821

Fletcher LE (2008) Cooperative signaling as a potential mechanism for cohesion in a gregarious sawfly larva, *Perga affinis*. Behavioral Ecology and Sociobiology 62:1127-1138

Fordyce JA (2003) Aggregative feeding of pipevine swallowtail larvae enhances hostplant suitability. Oecologia 135:250-257

Gallepp GW (1974) Behavioral ecology of *Brachycentrus occidentalis* Banks during pupation period. Ecology 55:1283-1294

Ghent AW (1960) A study of the group-feeding behavior of larvae of the jack pine sawfly, *Neodiprion pratti banksianae* Roh. Behaviour 16:110-147

Grison P (1948) Effet du groupement sur la croissance des chenilles du bombyx cul-brun (*Euproctis phaeorrhaea* Don Lep-Liparidae). Comptes Rendus des Seances de La Societe de Biologie et de Ses Filiales 142:610-612

Hamilton WD (1971) Geometry for selfish herd. Journal of Theoretical Biology 31:295-311

Hughes WOH, Oldroyd BP, Beekman M, Ratnieks FLW (2008) Ancestral monogamy shows kin selection is key to the evolution of eusociality. Science 320:1213-1216

Hunter AF (2000) Gregariousness and repellent defences in the survival of phytophagous insects. Oikos 91:213-224

Kalin M, Knerer G (1977) Group and mass effects in diprionid sawflies. Nature 267:427-429

Karban R (1998) Caterpillar basking behavior and nonlethal parasitism by tachinid flies. Journal of Insect Behavior 11:713-723

Knapp R, Casey TM (1986) Thermal ecology, behavior, and growth of gypsy moth and Eastern tent caterpillars. Ecology 67:598-608

Lawrence WS (1990) The effects of group size and host species on development and survivorship of a gregarious caterpillar *Halisidota caryae* (Lepidoptera: Arctiidae). Ecological Entomology 15:53-62

Lyons LA (1962) The effect of aggregation on egg and larval survival in *Neodiprion swainei* Midd. (Hymenoptera: Diprionidae). The Canadian Entomologist 94:49-58

Morrow P, Bellas T, Eisner T (1976) *Eucalyptus* oils in the defensive oral discharge of Australian sawfly larvae (Hymenoptera: Pergidae). Oecologia 24:193-206

Ouedraogo RM, Goettel MS, Brodeur J (2004) Behavioral thermoregulation in the migratory locust: a therapy to overcome fungal infection. Oecologia 138:312-319

Reader T, Hochuli DF (2003) Understanding gregariousness in a larval Lepidopteran: the roles of host plant, predation, and microclimate. Ecological Entomology 28:729-737

Reavey D (1993) Why body size matters to caterpillars. In: Stamp N, Casey T (eds) Caterpillars: Ecological and evolutionary constraints on foraging. Chapman and Hall, New York, pp 248-279

Ruxton GD, Sherratt TN (2006) Aggregation, defence and warning signals: the evolutionary relationship. Proceedings of the Royal Society B-Biological Sciences 273:2417-2424

Schlegel HG (1992) General Microbiology, 7th edn. Cambridge Uinversity Press, Cambridge

Seymour R (1974) Convective and evaporative cooling in sawfly larvae. Journal of Insect Physiology 20:2447-2457

Smith DR (1993) Systematics, life history, and diversity. In: Wagner M, Raffa KF (eds) Sawfly life history adaptations to woody plants. Academic Press, Inc., San Diego, pp 3-32

Stamp NE, Bowers MD (1990) Body temperature, behavior and growth of early spring caterpillars. Journal of Lepidopteran Society 44:143-155

Stamp NE, Casey T (1993) Caterpillars: Ecological and evolutionary constraints on foraging. Chapman and Hall, New York

Tostowaryk W (1972) Effect of prey defense on functional response of *Podisus modestus* (Hemiptera: Pentatomidae) to densities of sawflies *Neodiprion swainei* and *N. pratti banksianae* (Hymenoptera: Neodiprionidae). Canadian Entomologist 104:61-69

Tsubaki Y (1981) Some beneficial effects of aggregation in young larvae of *Pryeria sinica* Moore (Lepidoptera, Zygaenidae). Researches on Population Ecology 23:156-167

Vulinec K (1990) Collective security: Aggregation by insects in defense. In: Evans L, Schmidt J (eds) Insect Defenses. State University of New York Press, Albany, pp 251-288

Whitehouse MEA, Lubin Y (2005) The functions of societies and the evolution of group living: spider societies as a test case. Biological Reviews 80:347-361

Wilson EO (1971) The Insect Societies. Belknap Press of Harvard University Press, Cambridge

Wilson EO, Holldobler B (2005) Eusociality: Origin and consequences. Proceedings of the National Academy of Sciences of the United States of America 102:13367-13371

Wrona FJ, Dixon RWJ (1991) Group-size and predation risk - a field analysis of encounter and dilution effects. American Naturalist 137:186-201